UNIVERSITY OF ALBERTA

Synthesis of Novel Cyclic Thiooligosaccharides

by

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ABSTRACT

Naturally occurring cyclic oligosaccharides, cyclodextrins (CDs), have been extensively studied and found numerous applications originating from their unique ability to form inclusion-complexes toward a large variety of substances. It has been of great interest to synthesize unnatural cyclic oligosaccharides that can incorporate different glycosidic linkages as well as varied numbers and types of monosaccharide units, not only the homopolymeric α-1,4-*O*-linked D-glucopyranoside residues found in the native CD family. A novel type of cyclic oligosaccharides, consisting of the 1,6-β-*S*-linked oligosaccharides **1, 2, 3** and **4**, have been designed and envisaged to be more stable towards acidic hydrolysis or enzymatic degradation, and they may possess different inclusion complex patterns when compared to their *O*-linked counterparts. These water-soluble carbohydrate-based *S, O*-mixed crown ethers are also expected to selectively bind toward transition-metal ions.

This thesis describes a methodology developed for the synthesis of novel cyclic β-1,6-*S*-linked glucopyranosides. The key intermediate was a linear thiooligosaccharide bearing an iodo group at C-6 of the non-reducing sugar and a 1-thioacetyl group at the anomeric center of the reducing end. The crucial macrocyclization step was achieved through base-promoted intramolecular S_N2 glycosylation in remarkably high yields (84-95%), and with well-controlled stereochemistry. The methodology was also successfully applied to prepare a disaccharide monomer in an attempted to synthesize cyclic β-1,4-*S*-linked glucopyranoses through polycondensation methodology.

^{1}H and ^{13}C NMR spectra of these cyclic oligosaccharides reveals their highly symmetrical structures. Their 3D structures generated by Insight III program shows the diameters of the cavities ranged from 1.1 to 8.4 Å. The metal complexation of cyclic thiooligosaccharides **2, 3** and **4** in water is examined by ESI-MS, and exhibited the strong binding with silver (I), nickel (II), copper (II), cobalt (II) and cadmium (II) ions. An NMR titration study with Ag (I) showed cyclic trisaccharide **2** and tetrasaccharide **3** could form stable 1:1 cyclic sugar/metal complexes while a 1:2 complex for pentasaccharide **4**.

Dedicated to my family

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LIST OF ABBREVIATIONS

Ac	acetyl
All	allyl
anal.	analysis
aq.	aqueous
Bn	benzyl
b	broad
Bu	butyl
Bz	benzoyl
calcd	calculated
CSA	camphor-10-sulfonic acid
d	doublet or day(s)
DBU	1,5-diazobicyclo[5,4,0]undec-5-ene
DMAP	4-(dimethylamino)pyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DTT	dithiolthreitol
eq.	equivalent
Et	ethyl
Gal	D-galactopyranose
Glc	D-glucopyranose
h	hour(s)
Hz	hertz
HMBC	heteronuclear multiple bond correlation
HMPA	hexamethylphosphoramide
HMQC	heteronuclear multiple quantum coherence

HR-ESMS	high resolution electrospray mass spectrometry
I	iodo
J	coupling constant
m	multiplet
m/z	mass to charge ratio
Me	methyl
mg	milligram(s)
MHz	megahertz
min	minute(s)
mL	milliliter(s)
mol	mole(s)
mmol	millimole(s)
MS	mass spectrometry or molecular sieves
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
Ph	phenyl
ppm	parts per million
Py	pyridine
q	quartet
quant	quantitative
rt	room temperature
s	singlet
Satd	saturated
SSET	ethyl disulfide
t	triplet
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl

TDS	thexyldimethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSET	2-(trimethylsilyl)ethyl
Troc	2,2,2-trichloroethylformate
TROESY	transverse rotating frame nuclear Overhauser enhancement spectroscopy
Ts	*p*-toluenesulfonyl

Chapter 1

Introduction

1.1 Cyclic Oligosaccharides

Carbohydrates are the most abundant and disparate class of biological molecules in living systems [1,2]. They have been considered as energy sources, structural components, chemical mediators and receptors for binding bacteria, toxins, viruses and hormones. Saccharides found in nature are derived from simple molecules through gluconeogenisis [3] or carbon dioxide and water through photosynthesis. Oligosaccharides are usually covalently assembled from monosaccharides. The connectivity of these monosaccharide units is limited only by the number of available hydroxyl groups present on the molecules. This leads to a large variety of possible linear and branched structures. The structural diversity and complexity of carbohydrates are the key features that differentiate them from those of proteins and nucleic acids.

Despite the abundance and structural diversity of carbohydrates in nature, the number of naturally occurring cyclic oligosaccharides is rather limited [4,5]. Native cyclic oligosaccharides usually result from the action of bacterial enzymes on linear or branched oligosaccharides [6-8]. **Figure 1.1** shows some cyclic oligosaccharides isolated from natural sources [9-14]. Among these cyclic sugars, cyclodextrins (CDs) are undoubtedly the most important cyclic oligosaccharides in nature [15-25].

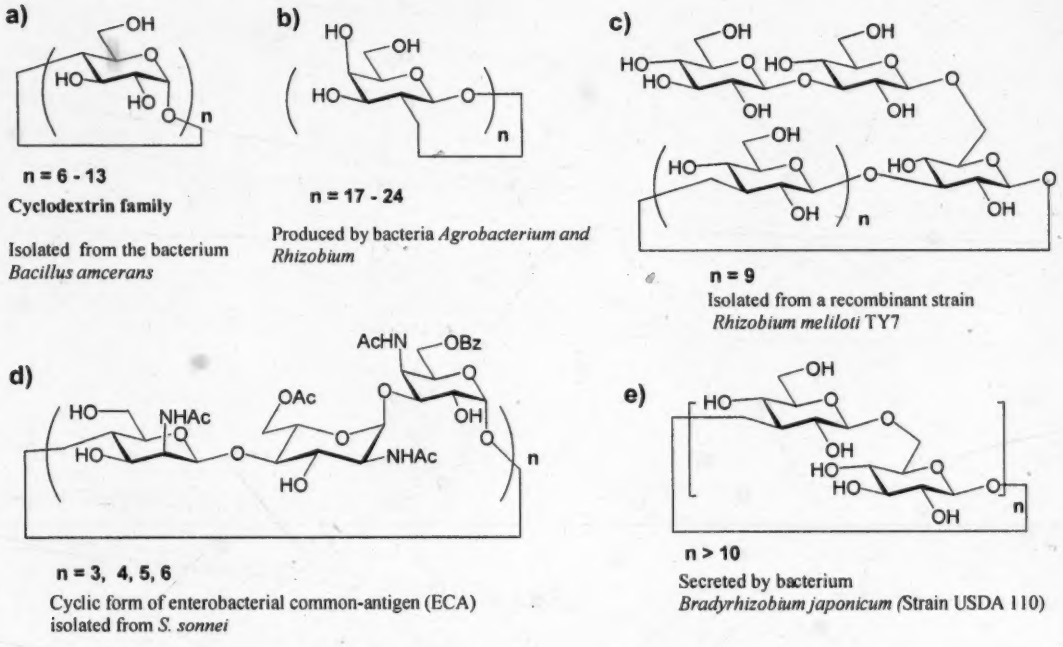


Figure 1.1 Cyclic oligosaccharides in nature.

1.1.1 Structural Features of Cyclodextrins

As widely used natural cyclic oligosaccharides, cyclodextrins were first isolated in 1891 from the bacterial digestion of starch, and it was not until the 1950s that the chemical structures of α-CD (n = 6), β-CD (n = 7) and γ-CD (n = 8) were finally confirmed **(Figure 1.2)**.

Cyclodextrins are non-reducing saccharides which are composed of six, seven, eight or more homogenous α-(1,4)-linked D-(+)-glucopyranose residues. X-ray analysis has clearly revealed that the D-glucopyranose units are in an undistorted $^4C_1$ chair conformation.

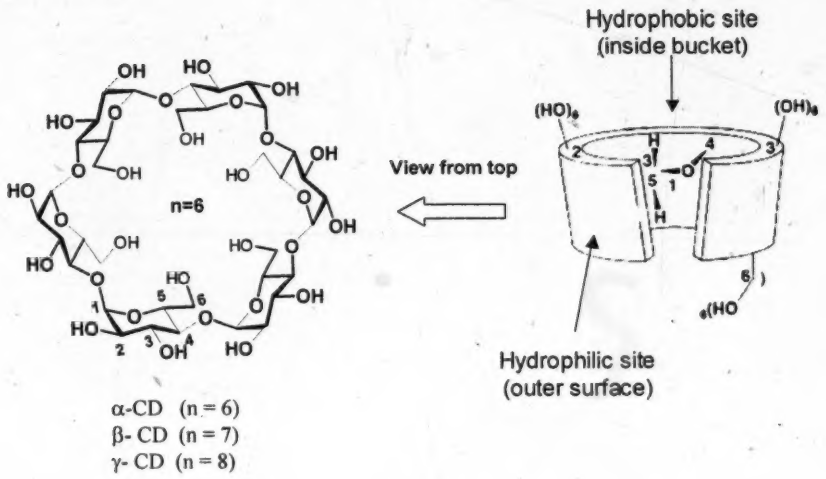


Figure 1.2 Structural features of cyclodextrins.

Cyclodextrins have truncated cone structures as shown in **Figure 1.2**. The secondary OH-2 and OH-3 of each glucopyranose unit are located at the more open top of the bucket, while OH-6 sits around the narrow rim. H-3, H-5 and the glucose ring oxygen atoms line the interior cavity whereas H-1, H-2 and H-4 are on the outer surface of the cyclic molecule. The strong hydrogen bonding between OH-2 and OH-3 of the adjacent units greatly limits the rotation of glucose units in these rather rigid cyclic molecules. The interior region of CD cavities is slightly hydrophobic compared to their hydrophilic outer surface.

The top and bottom diameters of the cavities are 4.7 and 5.3 Å for α-cyclodextrin, 6.0 and 6.5 Å for β-cyclodextrin, and 7.5 and 8.3 Å for γ-cyclodextrin, respectively. The depth of their tapered cavity is 7.9 Å [24].

1.1.2 Inclusion Complex Formation

The cyclodextrins are the first and probably the most important organic molecules found to have the ability to complex with other organic compounds. They can act as host toward a large variety of apolar aliphatic and aromatic substances. In aqueous solution, the slightly apolar cyclodextrin cavities are occupied by water molecules which are thermodynamically unfavored (polar-apolar interaction) and therefore can be readily displaced by appropriate guest molecules (**Figure 1.3**).

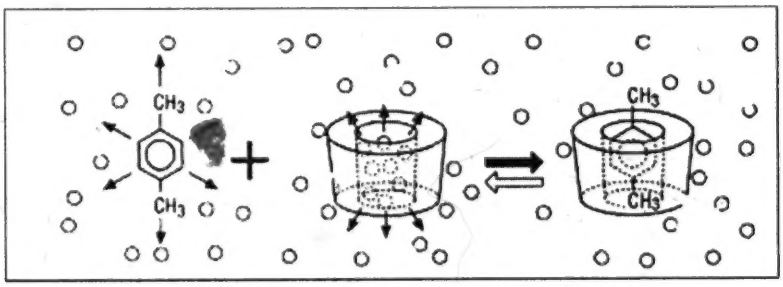


Figure 1.3 Schematic representation of inclusion-complexation formation (*p*-xylene is the guest molecule).

Van der Waals interactions (including dipole-dipole, dipole-induced dipole and induced dipoles) are the predominant attractive forces between a cyclodextrin host and a lipophilic substrate. The inclusion-complexation is best understood as a thermodynamic binding process as the formation of complex is the substitution of the high enthalpy water molecules.

Almost every class of organic compounds such as hydrocarbon, alcohol, amine, acid, naphthalene, etc. can be included in α-, β- and γ-cyclodextrins. **Table 1.1** lists some thermodynamic data of these cyclodextrin-complexes.

Table 1.1 Thermodynamic data of some representative cyclodextrin-substrate complexes.

Host	Guest	Log K	Δ G° (KJ mol^{-1})	Δ H° (KJ mol^{-1})	TΔS ° (KJ mol^{-1})	Method	Ref.
α	acetic acid	3.8±1.2	-21.8±6.7	-5.0±0.4	16.2	cal.	[26]
α	2-aminobenoic acid	5.0±1.3	-28.5±7.5	-1.3±0.4	26.2	cal.	[26]
α	4-bromophenol	2.84±0.01	-16.2±0.4	-29.8±1.4	-13.7±1.5	cal.	[27]
α	1,4-butanediol	0.89±0.02	-5.1±0.1	-11.7±0.4	-6.6±0.6	cal.	[28]
β	1-naphthaleneacetate	4.35±0.05	-24.8±0.3	-4.6±0.3	20.2	cal.	[29]
γ	4-[4(hydrophenyl)azo] benzoate	4.13±0.09	-25.7	-20.4±0.1	3.1	cal.	[30,31]
α	D-glucose	2.65±0.14	-15.1±0.8	-0.14±0.03	-31	cal.	[32]
β	L-proline	3.08	-17.5	5.0	22.2	uv.	[33]
β	hexane	---	1.9	-28±4	-29±4	sol.	[34,35]
α	cyclohexanol	1.76±0.01	-10.2	-13.8±0.2	-3.6±0.3	cal.	[36]

1) Complex stability constant (Log K), standard free energy (Δ G°), enthalpy (Δ H°) and entropy (TΔS °) for 1:1 complexation of various guests with natural cyclodextrins.
2) cal. = calorimetry; uv. = spectrophotometry; sol. = solubility measurement.

1.1.3 Applications of Cyclodextrins

Natural and chemically modified cyclodextrins have found numerous applications in many fields. It is well known that CDs can accelerate many kinds of organic reactions (hydrolysis of esters, oxidation, etc.) and exhibit remarkable selectivities in asymmetric reactions. The first example of a β-cyclodextrin accelerated reaction involved the hydrolysis of ethyl *p*-chloromandelate. In aqueous 1.32×10^{-3} M β-cyclodextrin, the rate of hydrolysis was accelerated 1.38 fold. This enhancement was the result of the substrate

substrate binding onto the cyclodextrin hydroxyl groups. Thus the complexed guest molecule is oriented favorably to undergo a transesterification to β-cyclodextrin. Some of representative reactions accelerated by cyclodextrins are listed in **Table 1.2.**

Table 1.2 Representative reactions accelerated by cyclodextrins.

Reaction	Substrates	Acceleration factor	Ref.
Hydrolysis of ester	Phenyl esters,	300	[37,38]
	Mandelic acid esters	1.38	[39-41]
Cleavage of amides	Penicillin	89	[42]
	N-acylimidazoles	50	[43]
Decarboxylation	Cyanoacetate anions	44.2	[44,45]
Oxidation	α-Hydroxylketones	3.3	[46]

Cyclodextrin-catalyzed reactions have many kinetic features similar to enzymatic reactions. In enzymology, CDs can serve as mimics of certain enzymes to help understand the enzymatic mechanisms [47-50].

In analytical chemistry, CDs are widely used for chiral separations. CDs can be either coated on the stationary phase in gas chromatography (GC) or immobilized on a suitable resin in HPLC [51]. In NMR spectroscopy, CDs have been utilized as chiral shift reagents to determine the optical purity [52].

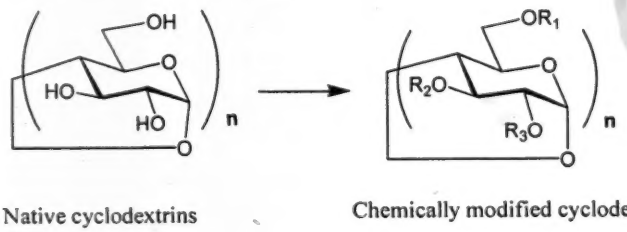
CDs have been found to be harmless toxicologically to humans [53-58], and they are often used as drug carriers in the pharmaceutical industry. The most common application of CDs is to enhance the solubility, stability and bioavailability of drug molecules [59-60]. The majority of drug molecules are poorly soluble in water, and their biological absorption is slow and often far from complete. In addition, many drugs are

also sensitive to oxidation, light and thermal decomposition but are stabilized as inclusion complexes.

In the food industry, CDs are used to suppress the odors and tastes of certain food additives and to make them more acceptable to consumers. β-CDs are used to specifically remove cholesterol from milk fat. In the cosmetics and pesticides industry [61], CDs are used to slow down the release of volatile perfumes and pesticides.

1.1.4 Chemical Modification of Cyclodextrins

Natural cyclodextrins have relatively low solubility, both in water and organic solvents, which limits their wide usage. Chemical modification of CDs has greatly enhanced their industrial applications. A large number of chemical modifications have been carried out on native CDs in order to enhance their solubility, to alter their inclusion characteristics, and to construct novel enzyme mimics.



Native cyclodextrins Chemically modified cyclodextrins

Figure 1.4 Chemical modifications of native cyclodextrins.

Substitutions of the hydroxyl groups on cyclodextrins with other kinds of functional groups are feasible (**Figure 1.4).** Methylated [62], hydroxyalkylated [63], acetylated [64], sulfated [65,66] and branched CDs (with glucosyl or maltosyl groups) can be produced in large scale. As a consequence of the different reactivities of hydroxyl

groups at 2, 3 and 6 positions on the glucopyranosyl ring of CDs, chemical modification can be carried out regioselectivity. The hydroxyl group at the 6-position is the most reactive one while OH-2 is slightly less reactive. OH-3 is the least reactive, probably because it is involved in the intramolecular hydrogen bonding network.

The chemical modification of CDs only involves the hydroxyl groups on the CD torus. The α-1,4-glucosyl linkages, the conformation of glucose units and the repeating units in CD derivatives are essentially identical to the natural CDs.

1.1.5 Chemical Synthesis of Cyclic Oligosaccharides

Apart from chemical modification of native CDs, total synthesis is the method of choice to construct unnatural cyclic oligosaccharides that can incorporate different inter-glycosidic linkages as well as varied numbers and types of monosaccharide units, not only the homopolymeric α-1,4-linked D-glucopyranoside units found in the native CD family.

The first total synthesis of α- and β-cyclodextrin was reported by Ogawa in 1987 in overall yields of 0.3% and 0.02%, respectively [67-68]. Starting from maltose, the bifuctionalized linear oligosaccharides were synthesized following standard carbohydrate methodology and then underwent intramolecular cyclizations to afford the desired products. As many efficient glycosylation methodologies were developed during last two decades, numerous other synthetic cyclic oligosaccharides have been reported. Some of these synthetic cyclic oligosaccharides are listed in **Figure 1.5** [67-77].

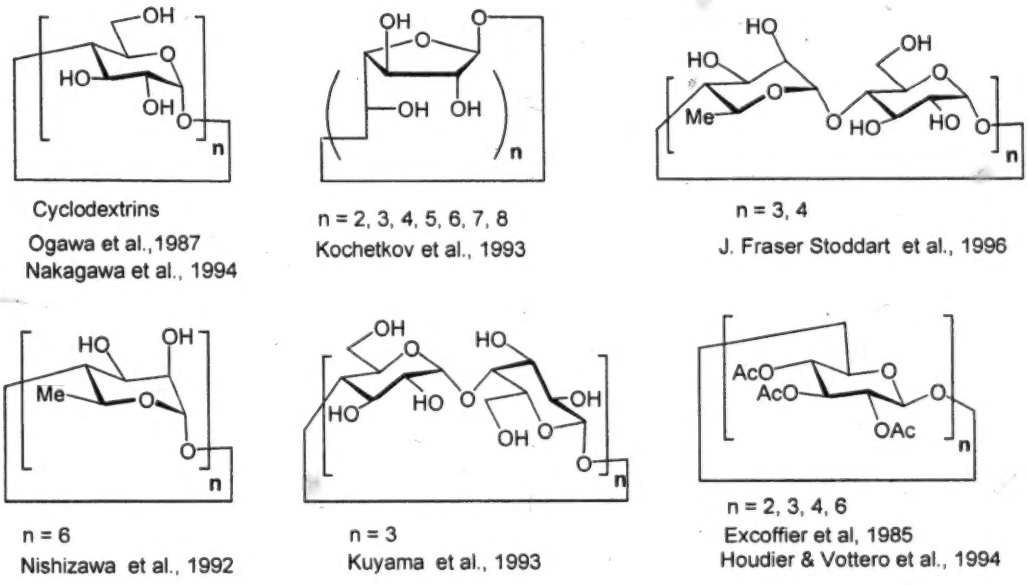

Cyclodextrins
Ogawa et al.,1987
Nakagawa et al., 1994
n = 2, 3, 4, 5, 6, 7, 8
Kochetkov et al., 1993
n = 3, 4
J. Fraser Stoddart et al., 1996
n = 6
Nishizawa et al., 1992
n = 3
Kuyama et al., 1993
n = 2, 3, 4, 6
Excoffier et al, 1985
Houdier & Vottero et al., 1994

Figure 1.5 Some of synthetic cyclic oligosaccharides.

There are quite a few challenges presented in the synthesis of cyclic oligosaccharides. First, a large number of glycosylation steps are usually involved. Secondly, the design and preparation of a linear oligosaccharide precursor bearing both a glycosyl acceptor and a glycosyl donor functional group on same molecule, requires a rather fine balance between appropriate stability and reactivity in the synthetic sequences. Finally, the overall yield is often limited by the macrocyclization step since there are no efficient methods available to fully control the final macrocyclization process.

1.1.5.1 Synthetic Strategies

Two main synthetic approaches (**Figure 1.6**) are usually applied to synthesize cyclic oligosaccharides: cycloglycosylation of a bifunctionalized linear oligosaccharide **1.6a** (approach A) and polycondensation of a disaccharide monomer **1.6b** (approach B) [78].

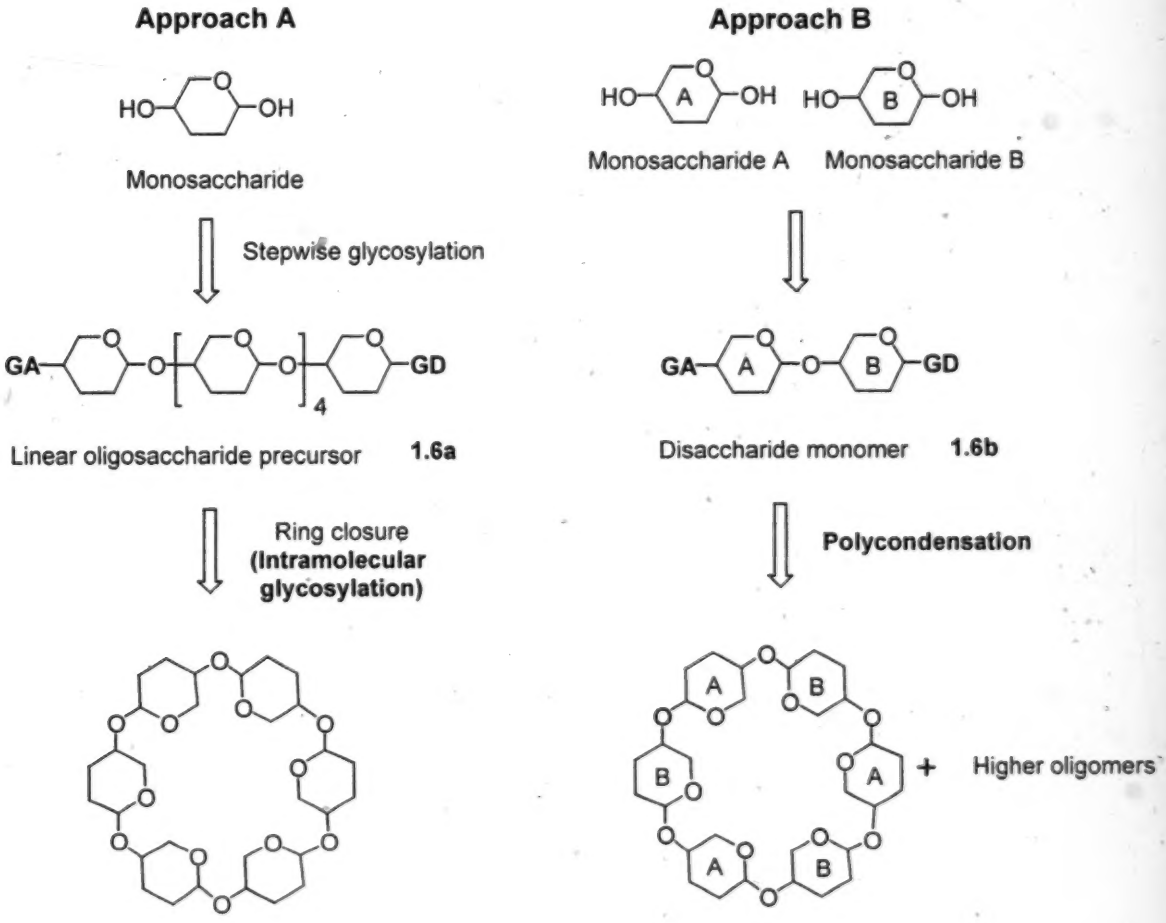


Figure 1.6 Schematic representation of the two main approaches to synthesize cyclic oligosaccharides (GA = glycosyl acceptor, GD = glycosyl donor).

1.1.5.1.a Cycloglycosylation of Bifunctional Linear Oligosaccharides

In this approach, a linear oligosaccharide is first synthesized from monosaccharides by multiple glycosylations (approach A in **Figure 1.6**). Many different kinds of monosaccharides can be potentially incorporated into different sequences. The two ends of the linear oligosaccharide are then modified to bear both glycosyl donor and acceptor functionalities to give precursor **1.6a.** Finally, the ring closure is achieved by an intramolecular glycosylation reaction.

Several techniques have been used to facilitate the macrocyclization process. High-dilution techniques [79,80] are the most often used methods to promote intramolecular glycosylation and suppress intermolecular reactions during the cyclization of a linear oligosaccharide precursor in approach A.

The conformation of the linear oligosaccharide precursor is crucial to the successful cycloglycosylation, since it determines if the glycosyl donor and acceptor on the same molecule are accessible to each other for the intramolecular glycosylation. The rigid group principle [81], structure-directed protocol [82,83] and template-directed method [84-87] have been applied to control the conformation of linear oligosaccharide precursor in order to promote the macrocyclization.

Since the synthesis of linear oligosaccharides involves multiple stepwise glycosylations, this process is laborious and the overall yield is usually low.

Alternatively, linear oligosaccharides can be obtained by the controlled cleavage of glucosidic bonds in native CDs. The resulting linear oligosaccharide can be further elongated by the addition of different saccharide units [88,89]. Cyclization of the newly formed linear precursor **1.7a** then generates novel cyclic oligosaccharides **(Figure 1.7).**

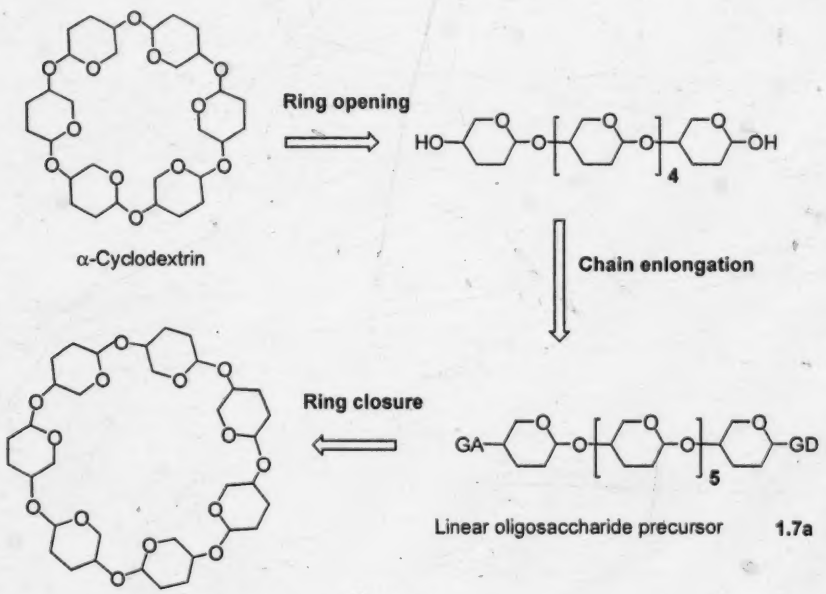


Figure 1.7 Cycloglycoslation of a modified linear oligosaccharide. Part of the linear oligosaccharide is prepared from opening the macrocyclic ring of CD.

1.1.5.1.b Polycondensation of Disaccharide Monomers

The key precursor in this approach is a disaccharide monomer **1.6b**, which is appropriately functionalized with a glycosyl donor and acceptor at its ends (approach B in **Figure 1.6**). The preparation of a disaccharide monomer is much less laborious than that of the synthesis of a long chain oligosaccharide. Several repeated glycosyl reactions (either in a sequential or a simultaneous fashion) are carried out, in one pot, between

disaccharide monomers during the assembling of cyclic oligomers. Although the yield of the macrocyclic step may be low, it saves a number of synthetic steps in the whole process, and the overall yield is usually acceptable.

This approach normally results in a range of cyclic oligomers of different sizes since the macrocyclization is not easily controllable. It provides a feasible way to construct a series of homo-polymeric cyclic sugars in a one-pot reaction [90]. The limitation of this approach is that only cyclic oligosaccharides composed of the same disaccharide repeating unit can be prepared.

1.2 Thiooligosaccharides

1.2.1 Thiooligosaccharides in Glycobiology

Thiooligosaccharides, in which at least one interglycosidic oxygen atom is substituted by a sulfur atom, have been extensively used in glycobiology as tools for understanding the interaction between oligosaccharides and glycosidases [91-97].

Carbohydrate-protein interaction [98-100] is a crucial process in the biosynthesis of natural oligo- and polysaccharides. It also plays an important role in transferring biological information in living organisms. It generally involves a carbohydrate-protein recognition and binding process. Fully understanding such interactions may ultimately lead to new concepts in enzyme engineering and glycotherapy. One versatile approach to investigate such interactions is utilizing enzyme resistant substrates to map the active site

of enzymes such as glycosyl-hydrolases. Thiooligosaccharides have been known to be excellent hydrolytically inert substrates that mimic various binding models and serve as potential inhibitors of glycanases. The sulfur atoms in thiooligosaccharides are less electronegative than oxygen atoms. They have lower affinity for protons and thus less susceptible to the conjugate acid formation, which is required for the transition state of hydrolysis.

The substrate recognition process in carbohydrate-protein interactions is primarily dependent on the overall conformation of the oligosaccharides. Compared to their O-glycoside counterparts, thiooligosaccharides are not only more resistant to enzymatic degradation, but can also be competitive inhibitors towards such enzymes. Moreover, the thioglycosidic linkages have a high degree of flexibility between glycosyl units. This kind of flexibility enables thiooligosaccharides to easily adapt their conformations to better fit in the catalytic sites on the proteins.

1.2.2 Synthesis of Thiooligosaccharides

There are very few classes of naturally occurring carbohydrates containing anomeric sulfur. Two examples are the various glucosinolates found in mustard oil [101] and the thioglycosides of lincomycin found in *Streptomyces* species (**Figure 1.8**) [102]. Recently, the increasing applications of thiooligosaccharides in glycobiology have stimulated the development of efficient synthetic methodologies for thioglycosylation [103-105].

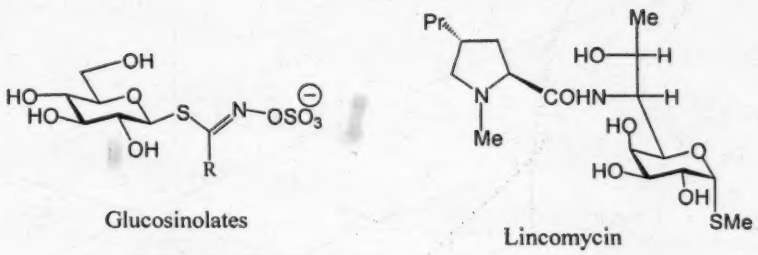
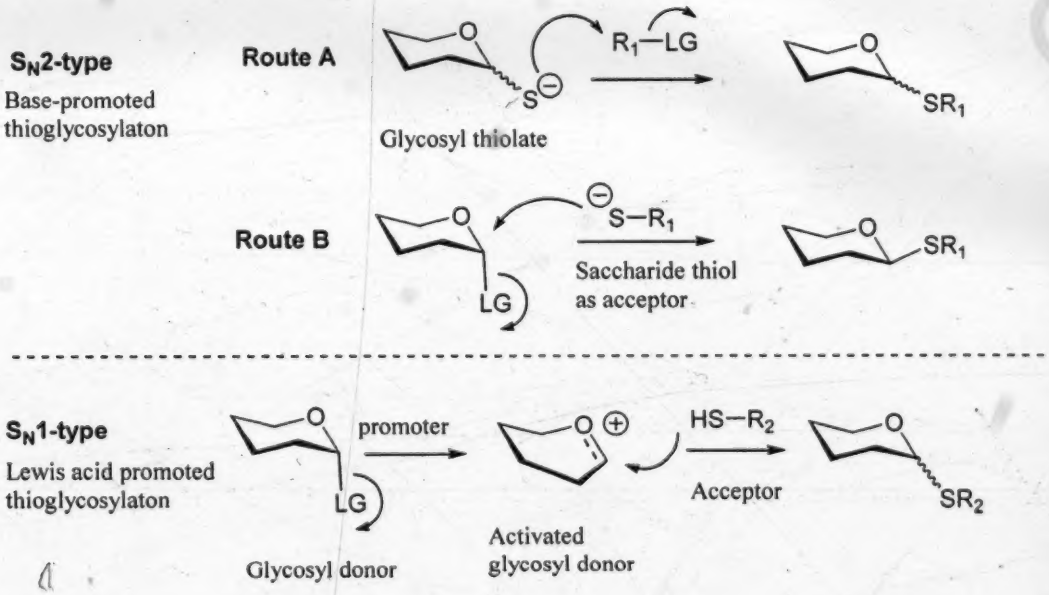


Figure 1.8 Naturally occurring thioglycosides.

There are two general strategies available for establishing thioglycosyl linkages (**Scheme 1.1**): A glycosyl thiol or thiolate directly displaces an anomeric leaving group in an S_N2 fashion. Alternatively, an anomeric leaving group could be displaced by a primary or secondary thiol and thiolate on a glycosyl acceptor.



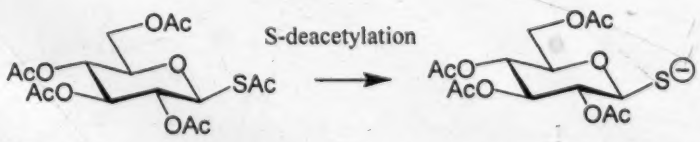
Scheme 1.1 General thioglycosylation methodologies (R_1 and R_2 are sugar moieties).

Similar to the O-glycosylation reactions, the alternative strategy involves a glycosyl oxoniumion is first generated from the cleavage of the anomeric leaving group, usually aided by a Lewis acid promoter. The activated glycosyl donor is subsequently captured by a mercaptan moiety, resulting in the formation of the desired thiooligosaccharide (S_N1-type). (**Scheme 1.1**).

1.2.2.1 S_N2-Type Base-Promoted Thioglycosylation

Under basic conditions, several types of glycosyl donors can be used. These include saccharides containing leaving groups (i.e. halides, tosylates and triflates), epoxide sugars, cyclic sulfate sugars and saccharides bearing an unsaturated keto moiety. Glycosyl thiols (in Route A) or saccharide thiols (in Route B) usually act as glycosyl acceptors in the thioglycosylation reactions (upper portion of **Scheme 1.1**).

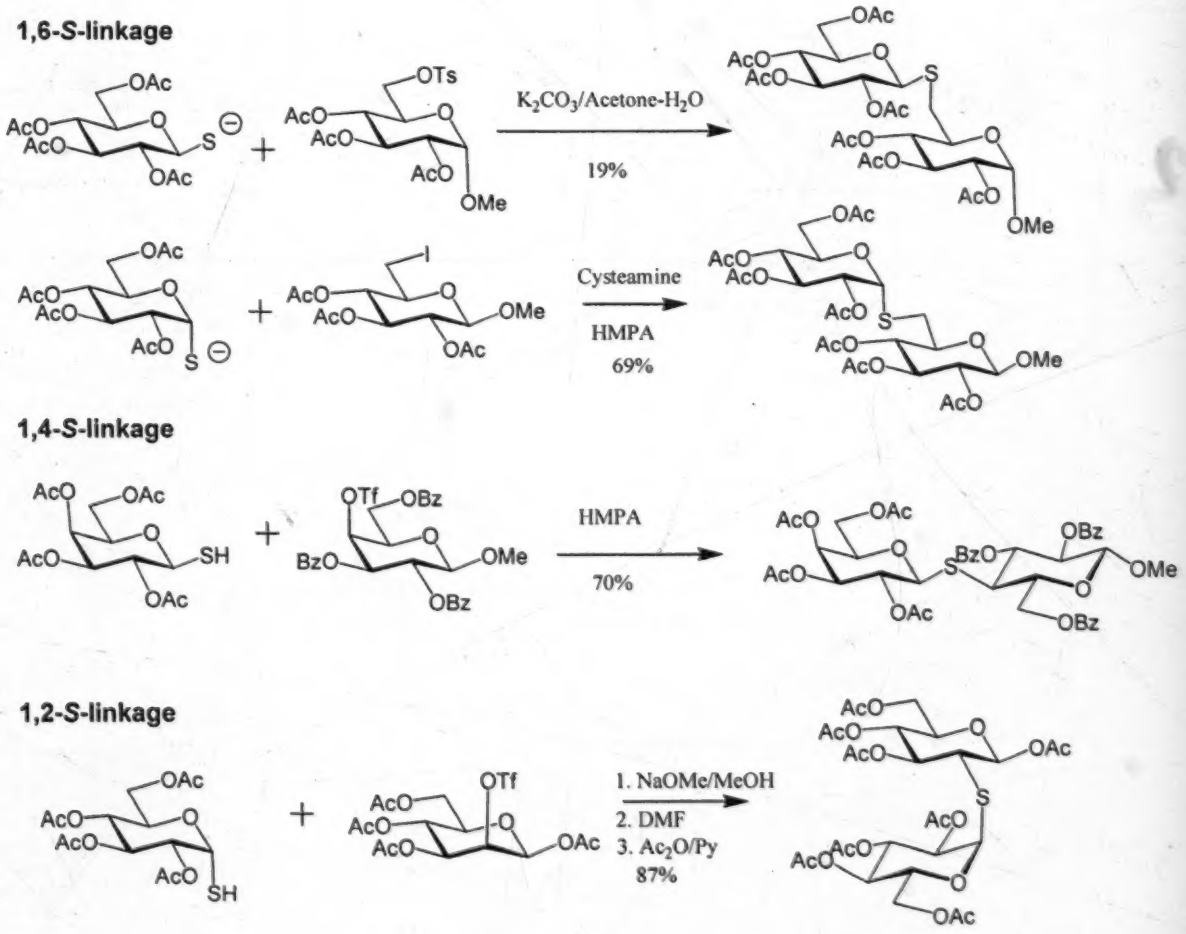
The most widely used method in the S_N2-type thioglycosylation is the displacement of the leaving group by an anomeric thiol or thiolate (Route A) since a glycosyl thiolate can be easily obtained from selective S-deprotection of a peracetylated 1-thioglycose **(Scheme 1.2)** [106-108]. On the other hand, the saccharide thiol or thiolate in Route B is often laborious to construct and usually cannot be used in large excess to promote the glycosidic bond formation.



Scheme 1.2 Selective S-deacetylation of peracetylated 1-thioglucose.

1.2.2.1a Halogens, Tosylates and Triflates as Leaving Groups

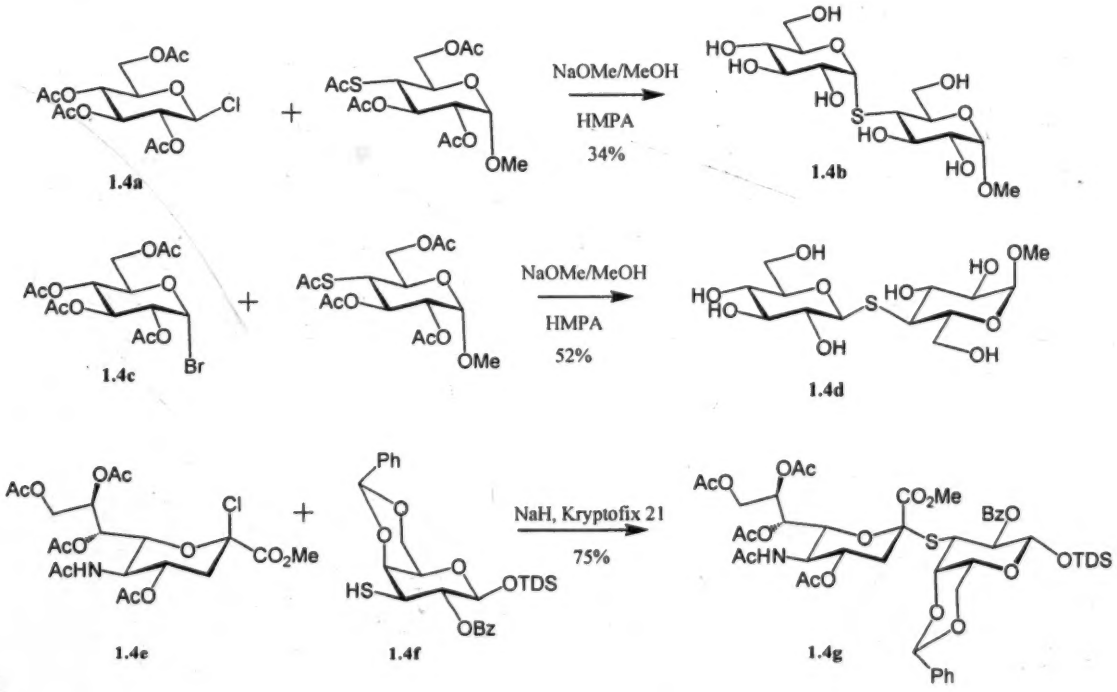
Halogens [109], tosylates [110] and triflates [111,112] are commonly used leaving groups when thioglycosylation is carried out with highly nucleophilic glycosyl thiolates.



Scheme 1. 3 Glycosylation between glycosyl thiols or thiolates and saccharides bearing halogens, tosylates or triflates as leaving groups.

In the construction of 1,6-*S*-linkages, even poor leaving groups at C-6 work well. Displacement of bromide and tosylate requires harsher conditions (higher temperature). Triflates often result in low yield due to elimination. Iodide has been proved to be the most effective leaving group for the establishment of 1,6-*S*-linkages. For other thioglycosylations involving secondary carbons, only triflates led to the formation of (1,2)-, (1,3)- and (1,4)-linkages. (**Scheme 1.3**)

Under basic conditions, anomeric chlorides and bromides also have been condensed with saccharide thiolates to introduce thio-linkages (**Scheme 1.4**). Usually this approach is not as efficient as the method mentioned above. Glycosyl bromides and

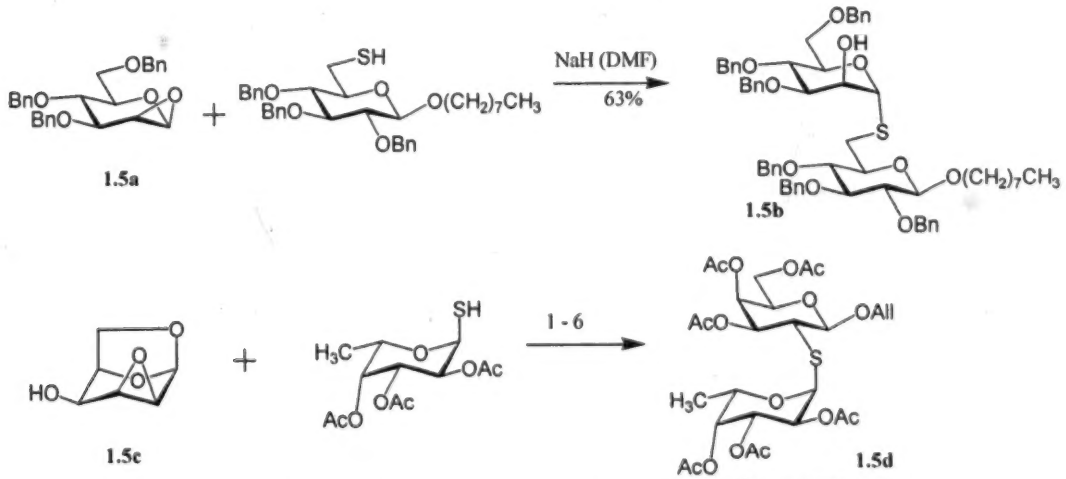


Scheme 1.4 Glycosyl halogens as glycosyl donors.

chlorides are relatively unstable under basic conditions, and side reactions such as elimination and hydrolysis make the overall yields relatively low. When a reactive β-glucopyranosyl chloride **1.4a** was employed to synthesize methyl 4-thiomaltoside **1.4b**, the yield was quite low (34%) **(Scheme 1.4)** [113]. 4-Thiocellobioside **1.4d** was also synthesized from glucosyl bromide **1.4c** in only a slightly better yield (52%). Displacement of the β-chloride of peracetylated neuraminic acid **1.4e** by a 3-thiogalatopyranoside derivative **1.4f** in the presence of NaH and crown-ether Kryptofix-21 gave the desired thioglycoside **1.4g** [114].

1.2.2.1b Epoxide Sugar as Donors

Reaction of the manno-1,2-epoxide **1.5a** with octyl 2,3,4-tri-*O*-benzyl-6-thiol glucopyranoside, generated a 1,6-*S*-linked disaccharide **1.5b** containing a mannose residue in 63% yield **(Scheme 1.5)** [115]. 2,3-Epoxides have also been used for the

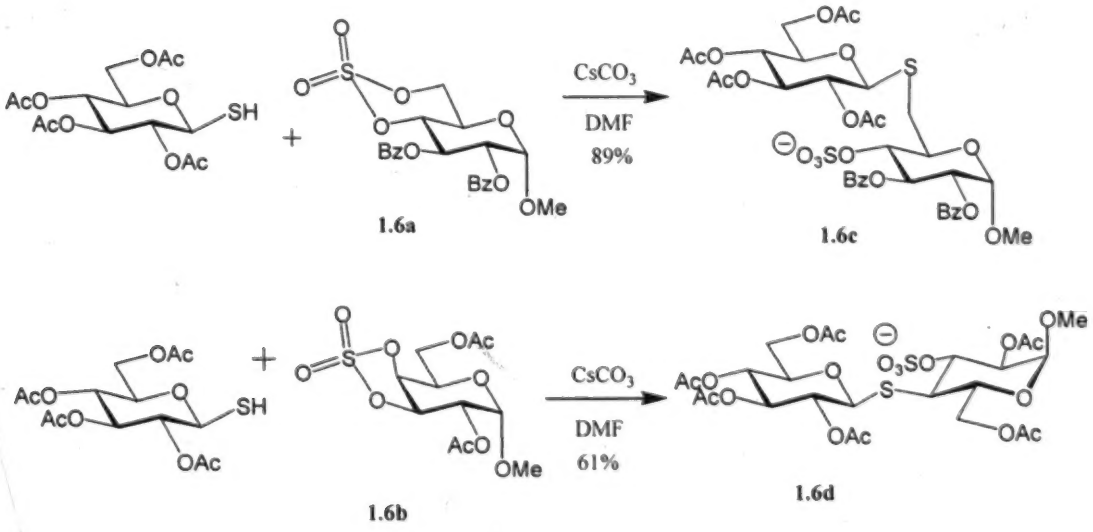


Scheme 1.5 Epoxide sugars as glycosyl donors.

nucleophilic attack. Oxirane-ring opening of the talopyroanose **1.5c** with α-fucosyl thiol afforded a fucosyl-anhydrogalactoside which after a series of reactions gave the disaccharide **1.5d** in an overall yield of 55% [116].

1.2.2.1c Cyclic Sulfates as Acceptors

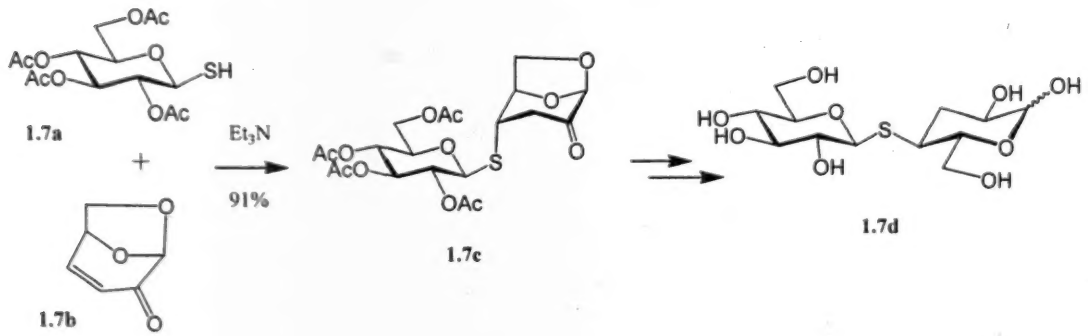
A highly stereoselective and simple method that uses a 4,6-cyclic sulfate to introduce the thio-linkage was developed by Santoyo-Gonzales et al. (**Scheme 1.6**) [117]. The reaction was promoted by cesium carbonate to afford *S*-linked disaccharide **1.6c** in 89% yield. Regioselective opening of 3,4-cyclic sulfate **1.6b** gave the desired 1,4-*S*-linked disaccharide **1.6d**.



Scheme 1.6 Thioglycosylation using cyclic sulfates as acceptors.

1.2.2.1d Michael Addition to Unsaturated Acceptors

Michael addition of glycosyl thiol **1.7a** with α,β-unsaturated sugar ketone **1.7b**, in the presence of Et_3N, gave the 1,6-anhydro disaccharide **1.7c** (**Scheme 1.7**) [118-120]. The ketone moiety in disaccharide **1.7c** was stereoselectively reduced by L-selectride, followed by a series of conventional treatments to afford the 1,4-*S*-linked disaccharide **1.7d** in 30% overall yield.



Scheme 1.7 Thioglycosylation by Michael addition to unsaturated sugar acceptors.

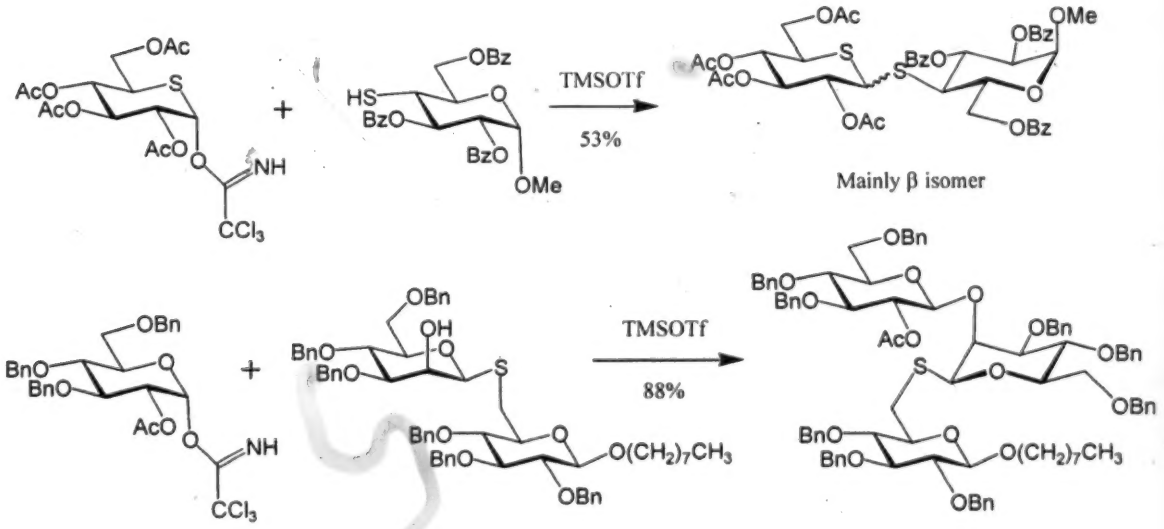
1.2.2.2 S_N1-Type Lewis Acid-Promoted Thioglycosylation

Thioglycosylations could be achieved under acidic conditions, between saccharide thiols and glycosyl donors such as glycosyl trichloroacetimidates, anhydro sugars and glycals.

1.2.2.2a Trichloroacetimidates as Donors

Activation of trichloroacetimidates involves hard Lewis acids as promoters ($BF_3{\cdot}Et_2O$ or TMS-triflate) (**Scheme 1.8**) [121-125]. It is believed that these promoters usually do not activate the product thioglycosides, so it is possible to prepare

thioglycosides from this type of donor [126]. The reaction normally involves an S_N1 mechanism and gives a mixture of α/β isomers. Although the stereochemical outcome is not easy to predict, the use of an acetylated α-trichloroacetimidate glucose can produce almost exclusively α- or β-linked disaccharide, depending on the acceptors and the reaction conditions used.

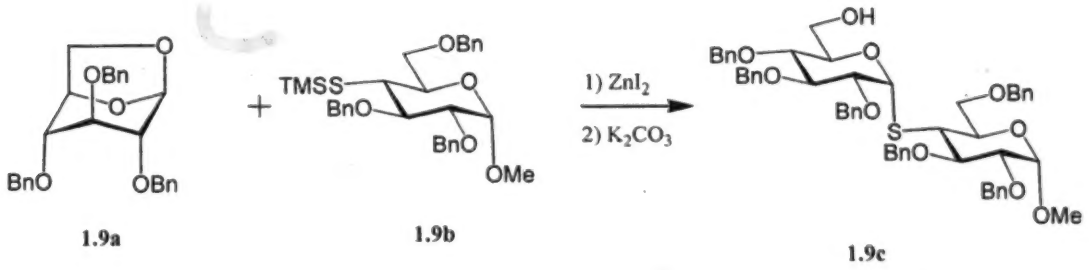


Scheme 1.8 Thioglycosylation using trichloroacetimidates as donors.

1.2.2.2b Anhydro Sugars as Donors

1,6–Anhydro sugars have been particularly useful as glycosyl donors since they generate disaccharides with a free OH-6, which can be utilized for further coupling reactions [127,128]. The coupling reaction between trimethylsilyl-4-thio-α-D-glucoside

1.9b and 1,6-anhydro sugar **1.9a** promoted by ZnI_2 gave maltose **1.9c** with high α-selectivity (**Scheme 1.9**).

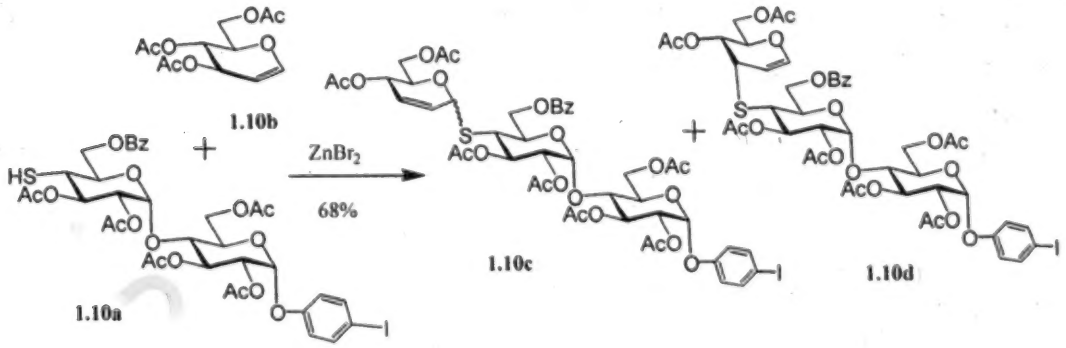


1.9a **1.9b** **1.9c**

Scheme 1.9 Thioglycosylation using anhydro sugars as glycosyl donors.

1.2.2.2c Glycals as Glycosyl Donors

The reaction of glycal **1.10b** with thiol **1.10a** involved an allylic displacement reaction (**Scheme 1.10**), giving 2,3-dideoxy-1-thioglycoside **1.10c** as the major product along with 3-S-alkyl-3-thiogycal **1.11d**, which resulted from the displacement of the 3-O-acetate in **1.10b**. The ratio between the two products was dependent on the substrate, the thio moiety and the Lewis acid used [129].

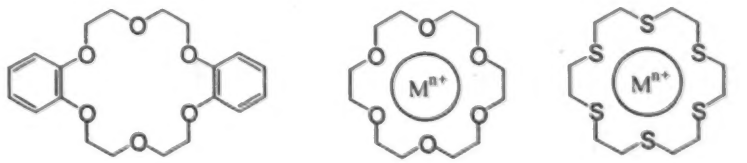


Scheme 1.10 Thioglycosylation using glycals as donors.

1.3 From Crown Ether to Crown Thiaether

Besides the well-known cyclodextrins, there are numerous other types of large-sized ring molecules found in nature, such as many cyclic peptides, marcolide antibiotics, porphyrin ring systems, etc. Research on the synthesis of macrocyclic compound has made significant progress over last few decades since the discovery of crown ethers.

In 1960s, Pedersen identified and synthesized a series of crown-like polyethers, which had the unusual property of complexing with alkali metal ions and alkaline earth metal ions [130-135]. Since then, macrocyclic polyethers ethers have found useful applications in a wide variety of fields such as organic synthesis (phase transfer catalysts), analytical chemistry (ion-selective electrodes), capture and separation of metal ions, resolution of optical isomers, etc. In 1987, Pedersen was awarded Nobel Prize for his discovery of crown ethers, and use of molecules with structure-specific interactions of high selectivity. **(Figure 1.9)**



Pedersen' crown ether (dibenzo-18-crown-6) | Crown ether | Thiacrown ether

Figure 1.9 Crown ethers and a thiacrown ether.

This discovery greatly stimulated interest in the investigation of the chemistry of all kinds of analogues of crown ethers. One of these analogues, thiacrown ethers, have been found to possess a very different metal complexing behavior (**Figure 1.9**). While

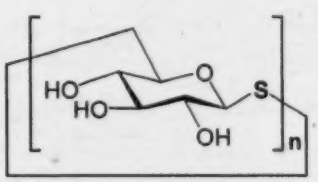
polyoxo crown ethers bind readily to main group cations, the sulfur atoms of polythio crown ethers coordinate to second- and third-row transition metal ions. Thiacrown ethers can stabilize the lower oxidation states of these ions. It was also found that their role as chelating agents for metals is more complicated than that of oxygen-based ethers. Thiacrown ethers were often observed to bridge cations, rather than chelate them.

Although thiaethers are generally regarded as poor ligands to transition metal centers, macrocyclic thiaethers readily bind to certain metal ions to form highly stable complexes, as result of the macrocyclic effect. The chemistry of crown ethers with late transition metal ions complements the well-established co-ordination chemistry of group I and II metal ions with polyoxo-crown ethers.

1.4 Objective and Scope of the Project

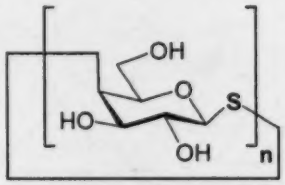
Despite the diversity of well-documented synthetic cyclic oligosaccharides, we observed that the synthesis of cyclic thiooligosaccharides, which contain exclusive sulfur glycosidic linkages, had never been explored. Inspired by the fascinating properties of cyclodextrins and crown ethers, we envisaged that cyclic thiooligosaccharides, as a new class of cyclic oligosaccharides, would be not only more stable towards acidic hydrolysis and enzymatic degradation than their *O*-linked counterparts, but also more interesting since the sulfur atoms in the ring system may bind selectively to certain metal ions. Replacement of glycosidic oxygen with a sulfur atom, which has larger atomic radius than oxygen atom, would also alter the geometry and hydrophobicity of the cavity and thus may lead to unique inclusion characteristics. This prompted us to synthesize cyclic thiooligosaccharides and explore their binding properties.

This thesis presents a methodology developed to synthesize a series of cyclic β-1,6-*S*-linked D-glucopyranosides containing two, three, four and five sugar residues (**Figure 1.10**). The structures of these compounds are essentially those of S, O-mixed crown ethers differing in the number of thioglucopyranoside rings. Preliminary studies on their complexation with transition metal ions are described. An attempt to apply the developed method to synthesis of cyclic β-1,4-*S*-linked-hexagalatoopyranside, a close analogue of thiocyclodextrin is also presented.



n = 2, 3, 4, 5

Cyclic 1,6-*S*-β-D-glucopyranose



n = 6

Cyclic 1,4-*S*-β–D-galactopyranose

Figure 1.10 Cyclic thiooligosaccharides.

Chapter 2

Synthesis of Cyclic *S*-linked β-1,6-Oligosaccharides

2.1 Introduction

A series of cyclic β-1,6-*S*-linked oligosaccharides **1, 2, 3** and **4** were chosen as our synthetic targets to develop a methodology for the synthesis of exclusively *S*-linked cyclic oligosaccharides **(Figure 2.1)**.

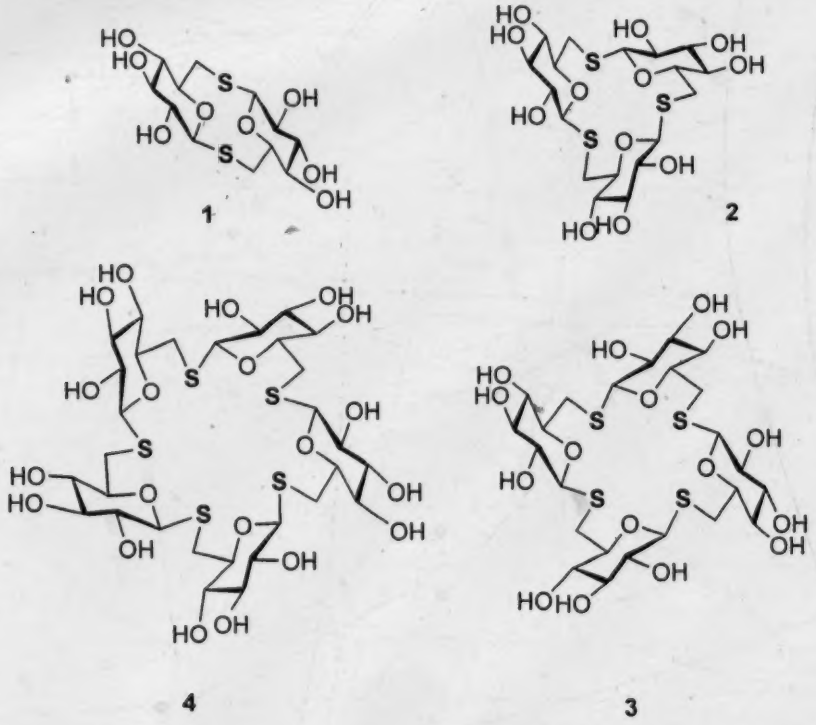


Figure 2.1 Synthetic targets: β-1,6-*S*-linked oligosaccharides.

In the literature a cyclic *O*-linked β-1,6-trisaccharide, an analogue of **2**, has been synthesized from its corresponding linear trisaccharide precursor [136]. Even a rigid

cyclic disaccharide with β-1,6-*O*-linkage, an analogue of **1**, was also isolated by Gagnaire et al [137]. Although the glycosidic *S*-linkage is different from the *O*-linkage in terms of bond length and tortion angle [138], we believe that our designed cyclic thiooligosaccharides **1, 2, 3** and **4** containing flexible β-1,6-linkages should be structurally stable.

In contrast to the small cyclic oligosaccharides joined by β-1,6-linkages, cyclization of a linear oligosaccharide with (1,2)-, (1,3)-, or (1,4)-glycosyl linkage usually requires at least five or six monosaccharide units. Therefore we decided to start our project with the assembly of a linear trisaccharide with β-1,6-linkage and focus on the investigation of the crucial macrocyclization step. Although our investigation on the macrocyclization step was originally planed on β-1,6-linked sugars, the methodology developed would also be expected to apply to the other linkages. More details will be discussed in Chapter **4**.

2.2 Synthetic Strategy

The strategy we employed to synthesize the target cyclic β-1,6-*S*-linked oligosaccharides includes: (1) Stepwise synthesis of linear thiooligosaccharides; (2) Functionalization of two ends of the linear thiooligosaccharides with a suitable glycosyl donor and a thioglycosyl acceptor; (3) Macrocyclization catalyzed either by Lewis acid, or through an S_N2-like displacement promoted under basic conditions **(Figure 2.2)**.

The key macrocyclization step, an intramolecular thioglycosylation on a linear thiooligosaccharide, has never been explored in carbohydrate chemistry. Synthesis of cyclic thiooligosaccharides presents more challenges compared to that of the regular *O*-linked cyclic sugars. The sulfur atoms in the thiooligosaccharides are susceptible to the thiophilic reagents and oxidative conditions, which would lead to the deconstruction of existing *S*-linkages. The nucleophilicity of sulfur atoms disqualifies many traditional protecting groups commonly used in carbohydrate chemistry. It also limits the choices of thioglycosyl donors and acceptors to very few of those available for the synthesis of linear thiooligosaccharides.

Moreover, the incompatibility between sulfur functionality and catalytic hydrogenolysis would complicate the use of benzyl ethers as protecting groups. Therefore, acetates or benzoates were selected to protect the hydroxyl groups that are not undergoing chemical modifications. Although Zemplèn deacetylation would easily provide the desired fully unprotected cyclic sugars, it also requires that synthetic steps should avoid harsh basic conditions. This leaves fewer choices for the orthogonal protecting groups for the functionalities at two ends on the linear thiooligosaccharide.

Two synthetic approaches were originally considered as shown in **Figure 2.2**. The target molecules could be derived from two possible linear precursors **2.2a** and **2.2b**, different from each other in the position of SH nucleophile and leaving group.

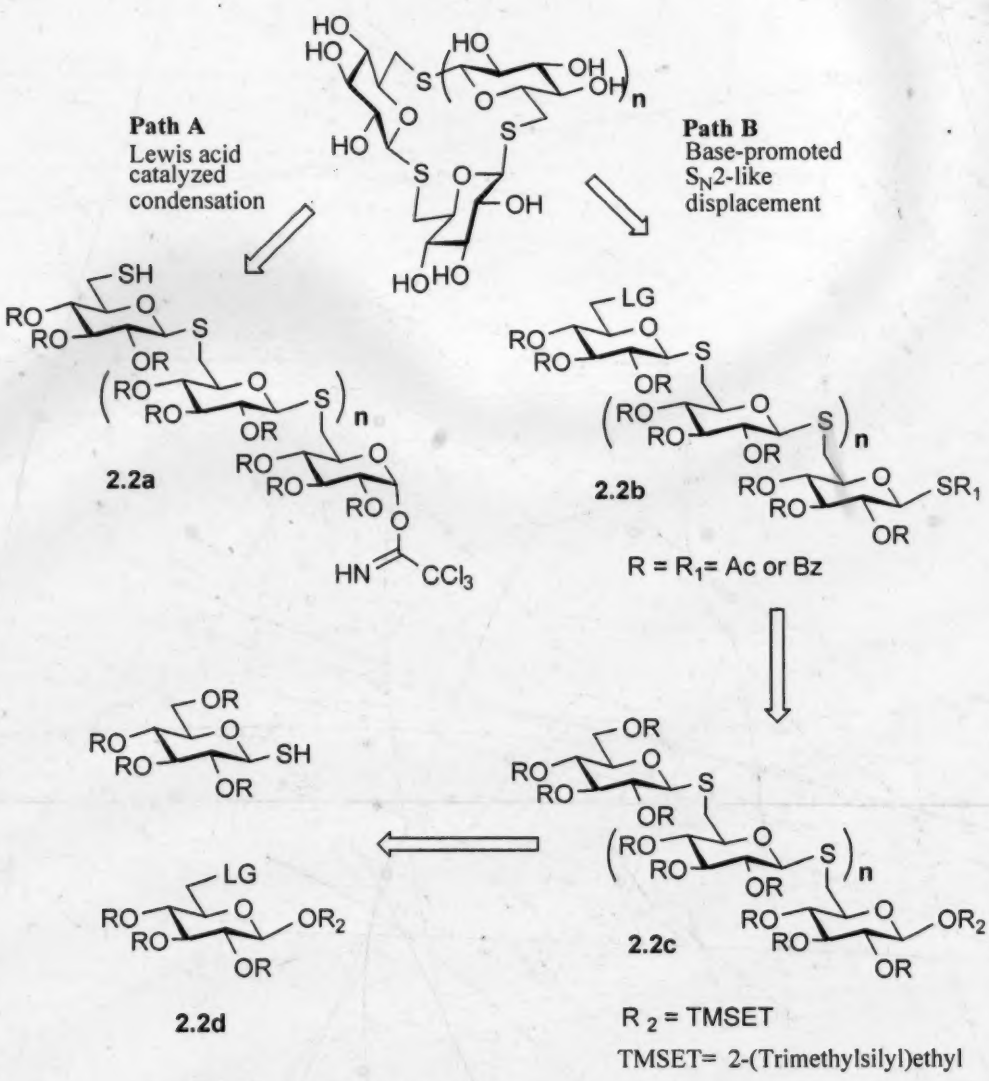


Figure 2.2 Retrosynthetic analysis of target molecules.

2.2.1 Approach A: Lewis Acid Catalyzed Condensation

Linear thiooligosaccharide **2.2a**, bearing a 6-SH group at the non-reducing end and a trichloroacetimidate at the anomeric position, was expected to undergo an

intramolecular glycosylation to afford the desired cyclic sugar using a Lewis acid as a promoter. However, we anticipated a number of potential problems with this approach.

Glycosyl trichloroacetimidates are usually used immediately after preparation. To prepare intermediate **2.2a**, a thioacetate group needs to be installed at the primary position of the non-reducing end before introducing the trichloroacetimidate at the other end. A thioacetate group at the primary position is often installed by substitution of a leaving group such as Br, Cl or tosylate, by potassium thioacetate. Introduction and activation of trichloroacetimidate requires both acidic and basic conditions, an orthogonal protecting group other than acetate is therefore desired for the SH-6 in **2.2a**. To the best of our knowledge, none of the available protecting groups meet the above requirement.

Moreover, trichloroacetimidate **2.2a** would also potentially lead to various elimination products and a side product orthoester. The stereochemical outcome of glycosylation is not easy to predict or control. Difficulties were anticipated in the purification of anomeric isomers and often optimization of conditions is necessary for each glycosylation. All of these potential problems promoted us to concentrate our efforts on the second approach described in Section **2.2.2**.

2.2.2 Approach B: Base-Promoted S_N2-Like Displacement

The other possible linear thiooligosaccharide **2.2b** possesses an anomeric thiol or thiolate at the reducing end and a leaving group at C-6 of the non-reducing end of the

linear oligosaccharide. Under basic conditions, the anomeric thiolate would displace the leaving group to achieve the macrocyclization. Polymerization might be an important competing reaction.

Intramolecular thioglycosylation of the precursor **2.2b** with a 1-thiolate would give exclusively the β-1,6-*S*-linkage. In this case, 1-thioacetate was chosen as the reducing terminus on the basis of its ease of S-deacetylation to produce a nucleophilic thiol.

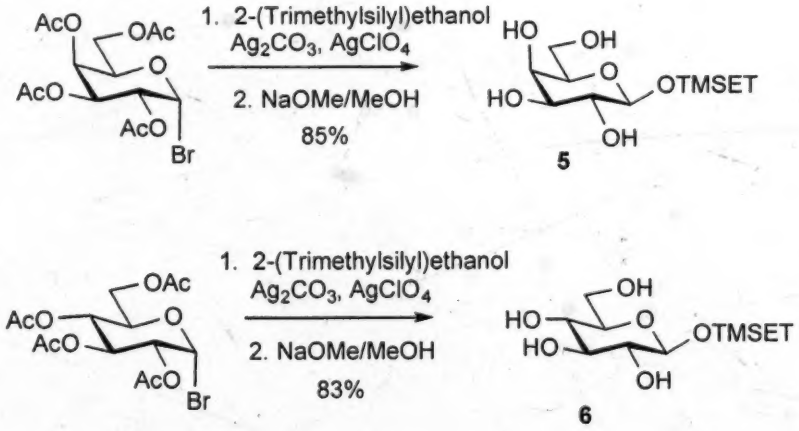
Retrosynthetically, the linear β-1,6-thiooligosaccharide **2.2c** can be derived from iterative coupling of a glycosyl thiolate and a building block **2.2d.** Although there are many methods available in the literature for the construction of a β-1,6-thiolinkage, the most effective way is the displacement of a 6-deoxy-6-iodo glycoside derivative with a 1-thiolate anion [139-141].

2-(Trimethylsilyl)ethyl (TMSET) was chosen to be the anomeric blocking group in both linear thiooligosaccharide **2.2c** and building block **2.2d**, because it is stable in the presence of a variety of reagents and can be selectively removed or transformed at conditions that are mild enough not to break intersaccharidic glycoside bonds [142,143].

2.3 Preparation of Building Blocks and β-1,6-Thioglycosyl Coupling

2.3.1 Exploratory Studies

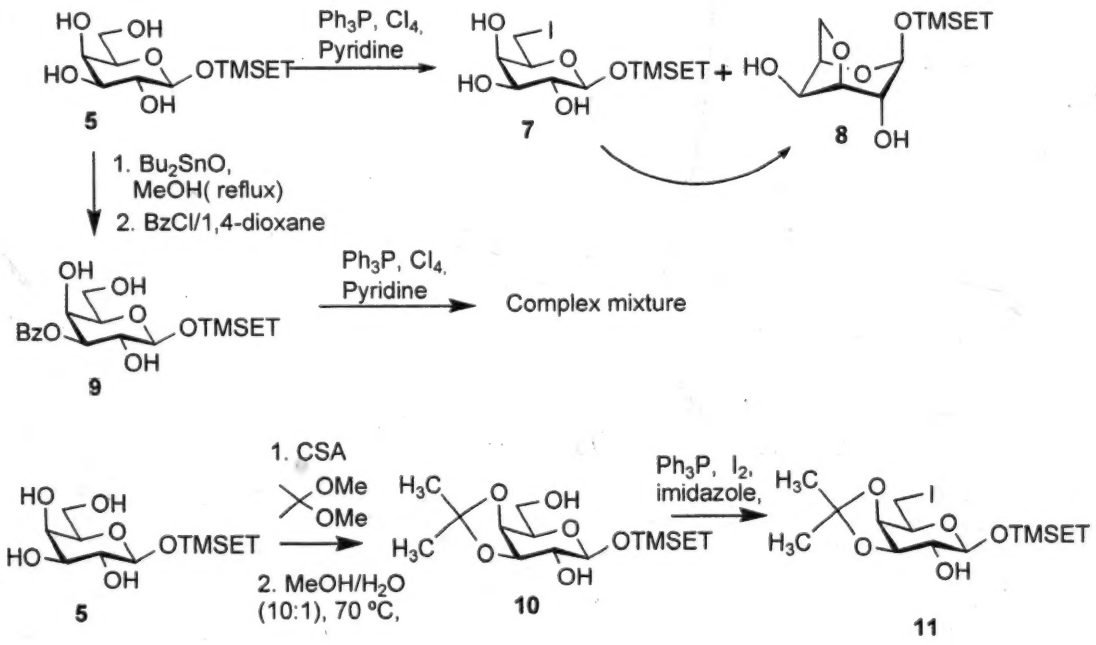
Two TMSET glycosides **5** and **6** were originally prepared (**Scheme 2.1**). TMSET galactopyranoside **5** was first conveniently synthesized on a large scale starting from 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide. Glycosylation in CH_2Cl_2, promoted by a mixture of silver percholorate and silver carbonate, gave an 85% yield of the expected product. The fully acetylated TMSET glycoside was treated under Zemplèn condition to provide **5**. The TMSET glucopyranoside **6** was synthesized in a similar manner.



Scheme 2.1 Preparation of TMSET glycosides **5** and **6.**

The selective halogenation of the primary hydroxyl group in unprotected sugars has been reported by Whistler et al. in almost quantitative yield [144]. Thus, the TMSET 6-deoxy-6-iodo glycoside **7** was designed as a building block (**Scheme 2.2**). TMSET galactopyranoside **5** was first treated with triphenylphosphine and carbon tetraiodide in pyridine following the procedure described by Whistler et al. In our hands, this method only gave a low yield of the desired product **7**, along with a large amount of the anhydro sugar **8**, derived from the intramolecular displacement of 6-

iodide by OH-3. Varying the temperature and duration of the reaction did not lead to any improvement.



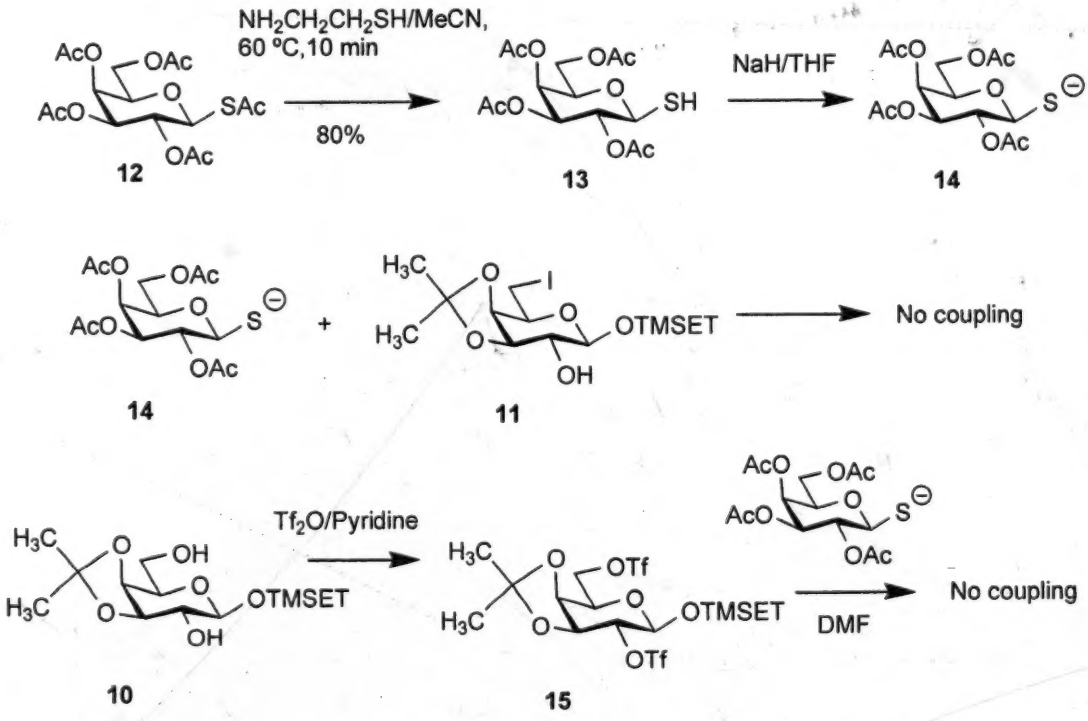
Scheme 2.2 Synthesis of preliminary building block **11.**

In an attempt to block the intramolecular displacement reaction, we decided to protect OH-3. The OH-3 of TMSET galactopyranoside **5** was thus selectively protected as the benzoate in two successive steps [145], as described in **Scheme 2.2.** The resulting compound **9** was then subjected to iodination. To our disappointment, a complex mixture was obtained.

At this point, we realized that the presence of unprotected hydroxyl groups in **5** may have complicated the iodination reaction. Consequently, both OH-3 and OH-4 were protected as the 3,4-*O*-isopropylidine acetal in a one-pot reaction (78%). The

subsequent iodination of the resulting intermediate **10** provided the desired **11** in high yield (86%). The overall procedure for the preparation of the TMSET 3,4-isopropylidene galactopyranoside **11** was amenable to large-scale synthesis.

With enough building blocks in hand, our first thioglycosyl coupling was attempted between a glycosyl thiolate **14** and building block **11** in DMF. Glucosyl thiolate **14** was prepared in two sequential steps (**Scheme 2.3**). First, glucosyl thiol **13** was generated by selective deacetylation of the thioacetate with a weak nucleophile, cysteamine ($H_2NCH_2CH_2SH$) [146]. In this process cysteamine also served as a



Scheme 2.3 Attempted thioglycosylation with building block **11.**

reducing agent to prevent the atmospheric oxidation of the glycosyl SH group. Treatment of **13** with 1.1 eq. of sodium hydride in THF provided the thiolate **14**. Partial deacetylation of **13** was observed under these basic conditions even at 0 °C.

To our surprise, reaction of thiolate **14** with iodide **11** (72 h, rt) did not give any of the desired product. It was initially thought that I-6 might not be a very strong leaving group in this case. Therefore, compound **15** with triflates at C-2 and C-6, was synthesized quantitatively from **10** and subjected to the coupling conditions. The reaction produced a complex mixture but still no coupling product was detected by ESI-MS. Guided by these results, we suspected that the 3,4-*O*-isoproperlidene on the galactopyranoside might block the backside of the sugar, thereby preventing an S_N2 reaction (**Figure 2.3**).

After a careful examination of the literature examples, we recognized that most of successful cases in the assembling of 1,6-*S*-linkages were carried out with glucopyranosides as glycosyl donors. We therefore decided to employ TMSET glucopyranoside, instead of galactopyranoside, as the building block to synthesize cyclic β-1,6-thiooligosaccharides.

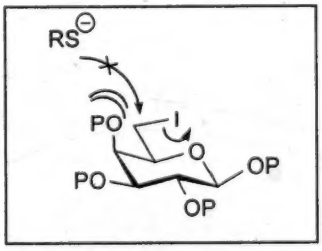
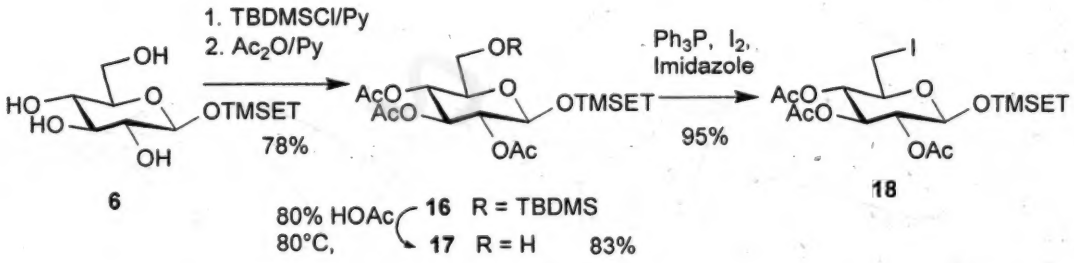


Figure 2.3 Accessibility of incoming nucleophile was sterically hindered when using galactopyranosides as thioglycosyl donors.

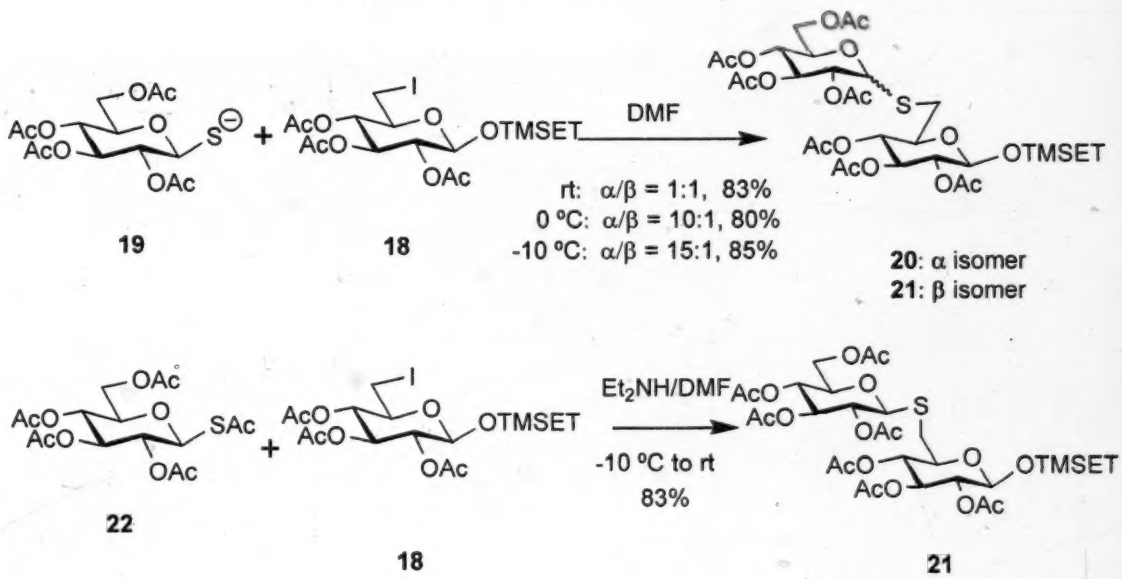
2.3.2 Synthesis of Building Blocks and Thioglycosyl Coupling

The 6-OH group of TMSET glucopyranoside **6** was first regioselectively protected as a TBDMS ether followed by acetylation of the remaining of hydroxyl groups. Selective removal of the TBDMS group in **16** was carried out by treatment with 80% acetic acid in water. The anomeric TMSET glycoside was stable and the acetyl migration between OH-4 and OH-6 was suppressed under these conditions. The iodination was achieved in 95% yield by treating **17** with triphenyl phosphine, iodine and imidazole in toluene **(Scheme 2.4)** [147].



Scheme 2.4 Preparation of building block **18.**

With the new building block in hand, **18** was reacted with a glucosyl thiolate **19** in DMF at room temperature. This resulted in the formation of the desired thio-linked disaccharide in 83% yield (α/β ratio=1:1). A higher percentage of β-isomer was obtained when the reaction was carried out at lower temperature. The α-isomer could be removed by chromatography or re-crystallization **(Scheme 2.5)**.



Scheme 2.5 Preparation of β-1,6-*S*-linked disaccharide **21.**

Our previous experiment showed that 6-iodo glucopyranoside **18** was stable in DMF in the presence of diethylamine for over 6 h. This observation prompted us to explore *in situ* thioglycosylation, which was expected to minimize the risk of atmospheric oxidation of the anomeric SH group and improve the overall yield. Thus, a DMF solution of thioacetate **22** (1.2 eq.) and **18** (1 eq.) was treated with diethylamine. The desired β-1,6-*S*-linked disaccharide **21** was isolated in 83% yield.

The *in situ* coupling approach not only reduced the synthetic steps, but also played a crucial role in the synthetic planning of macrocyclization toward the cyclic thio sugars.

2.4 Synthesis of Linear β-1, 6-*S*-Linked Oligosaccharides

Having *S*-linked disaccharide **21** in hand, we intended to convert the anomeric TMSET group into an anomeric thioacetate, which could be used for the next round thioglycosylation in order to make the required longer thiooligosaccharides.

2.4.1 Conversion of 1-TMSET to 1-SAc *via* the Glycosyl Chloride

One short route available for this process is to turn the 1-TMSET group into a glycosyl chloride, which can be easily converted into a 1-thioactate through a simple S_N2 reaction. It has been reported [148] that reaction of TMSET glucosides **2.4a** with 1,1-dichloromethyl methyl ether/zinc chloride in chloroform provided the α-D-glucopyranosyl chlorides **2.4b** in 98% yield (**Figure 2.4**). This transformation relies on the ability of the anomeric oxygen of the TMSET glycoside to react with electrophilic reagents. The anomeric oxygen carries an increased electron density as the result of the capacity of silicon to stabilize a positive charge on a carbon in the β-position.

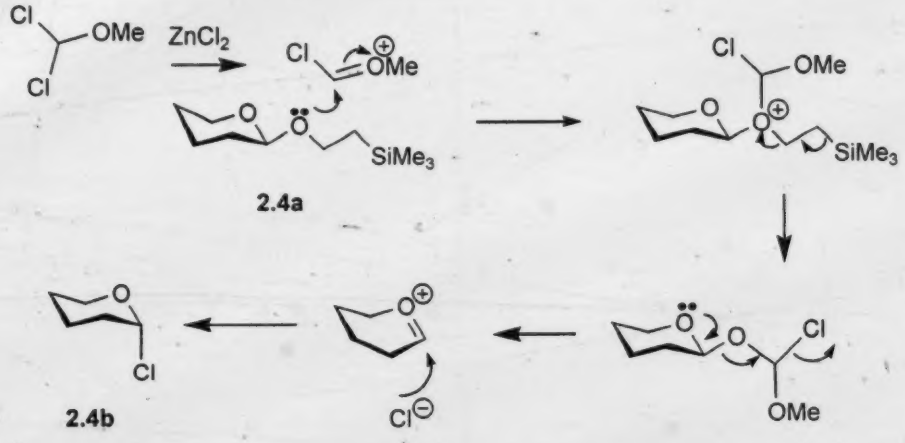
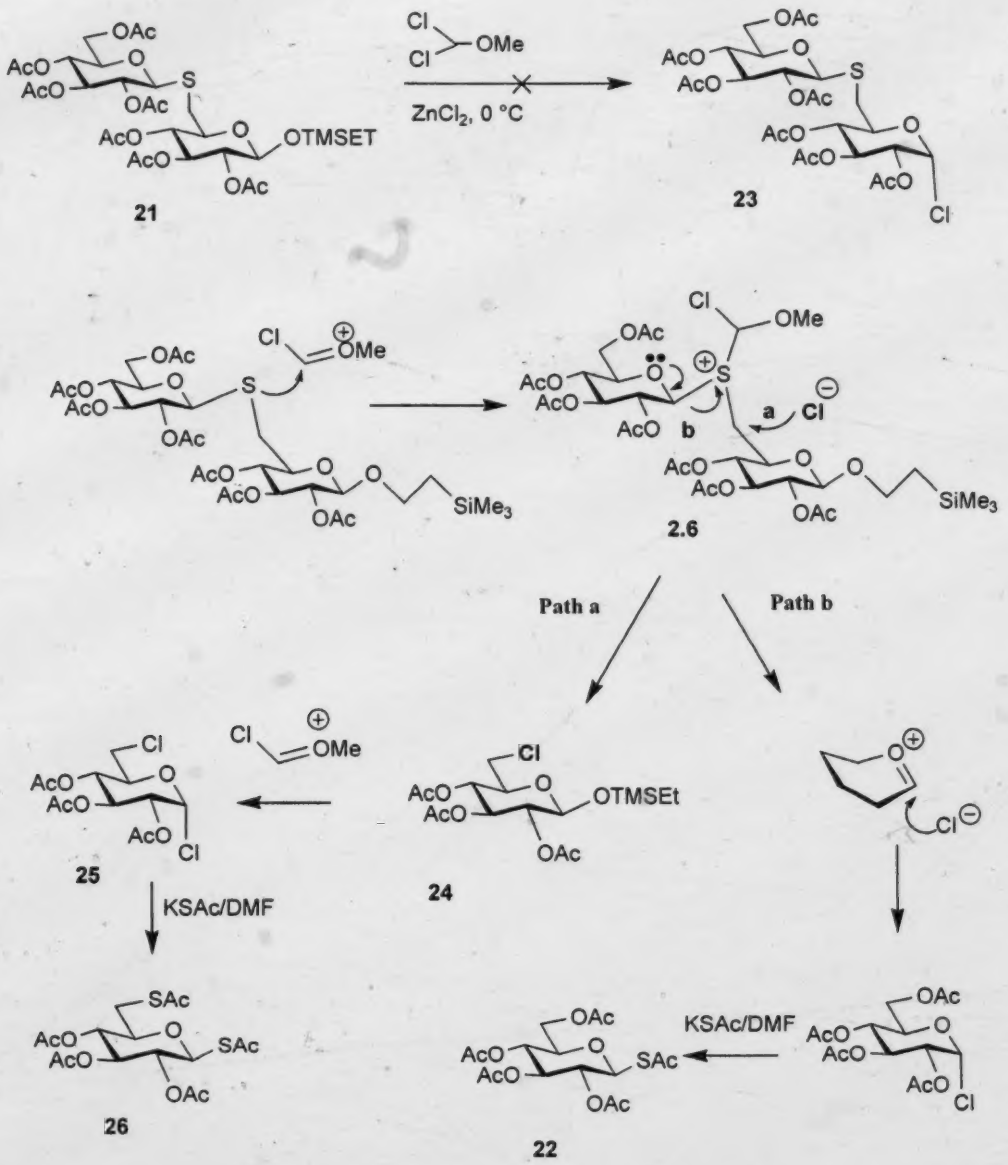


Figure 2.4 Transformation of a TMSET glycoside into a glycosyl chloride.

Although we understood the potential risk of a nucleophilic attack at chloromethylenoxymethyl cation by the sulfur atom in the interthioglycosyl linkage, we hoped that the reaction on anomeric oxygen would be faster. Unfortunately, treatment of



Scheme 2.6 Attempted conversion of TMSET disaccharide **21** to disaccharide thioacetate **30** *via* disaccharide chloride **23.**

disaccharide **21** under the same condition gave a mixture of products containing mostly monosaccharide fragments (detected by ESI/MS and monitored by NMR when the experiment was carried out in CD_2Cl_2). The crude mixture was washed and concentrated, and subsequently treated with KSAc in DMF. The main products obtained were glucopyranosyl thioacetate **22** and 1,6-dithioacetate **26** (**Scheme 2.6**).

It seems probable that the glycosidic sulfur atom reacted with the active species chloromethylenoxymethyl cation to form the sulfoxomiumion **2.6**. This active sulfoxomiumion may collapse *via* path **a** and **b** (**Scheme 2.6**). In path **a**, the C-6 in the α-position of **2.6** is attacked by a chloride ion to give a monosaccharide fragment, 2-(trimethylsilyl)ethyl 2,3,4-tri-O-acetyl-6-chloro-6-deoxy-β-D-glucopyranoside **24.** Further action on **24** by the active chloromethylenoxymethyl cation, could provide 1,6-dichloro glucopyranoside **25**. In path **b**, the lone pair electrons on the ring oxygen eliminate the anomeric sulfoxomiumion and produce a glycosyl oxomiumion, which is attacked by a chloride anion to afford the glycosyl chloride. This proposal was supported by the isolation of two fragments **26** and glycosyl thioacetate **22** after the reaction mixture was treated with potassium thioacetate.

The reaction was also carried out at varied lower temperatures (-10 °C, -20 °C and –40 °C) with the intention to suppress these side reactions. The results showed the reaction on sulfur atom still proceeded much more rapidly than the desired reaction at anomeric oxygen atom.

2.4.2 Conversion of 1-TMSET to 1-SAc *via* the Glycosyl Acetate

An alternative way to deblock the TMSET group was also attempted, in which the TMSET glycoside was treated with $BF_3 \cdot Et_2O$ / Ac_2O in order to generate glycosyl acetate (1-*O*-acyl sugar) shown in **Figure 2.5** [149]. The glycosyl acetate could then be further converted into a glycosyl bromide or chloride.

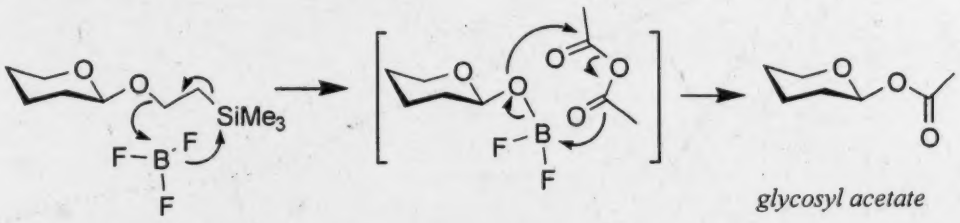


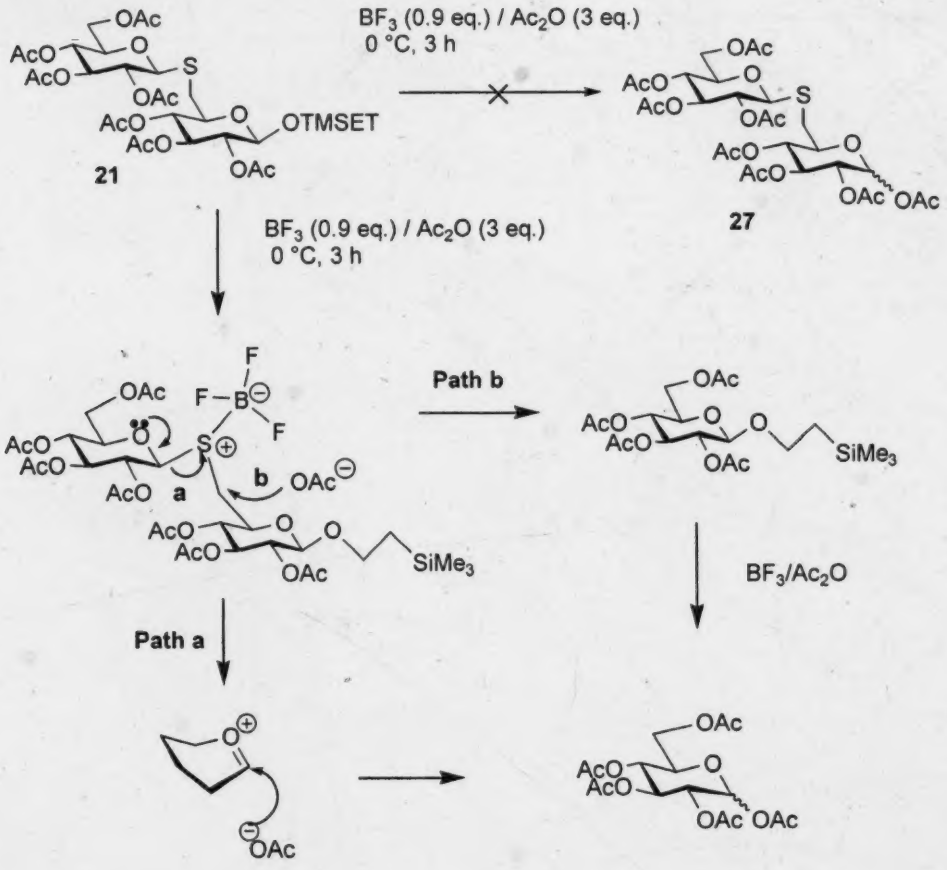
Figure 2.5 Conversion of the anomeric TMSET group into glycosyl acetate.

This approach also resulted in the cleavage of interglycosidic S-linkages. The sulfoxomiumion ion was generated from the nucleophilic attack at BF_3 by the glycosidic sulfur atom. The following collapse of the sulfoxomiumion ion could occur via two possible pathways, similar to the process described in **Scheme 2.6.** In this case an acetate anion was the electrophile to attack the sulfoxomiumion, affording the glucosyl acetate as the main product (**Scheme 2.7**).

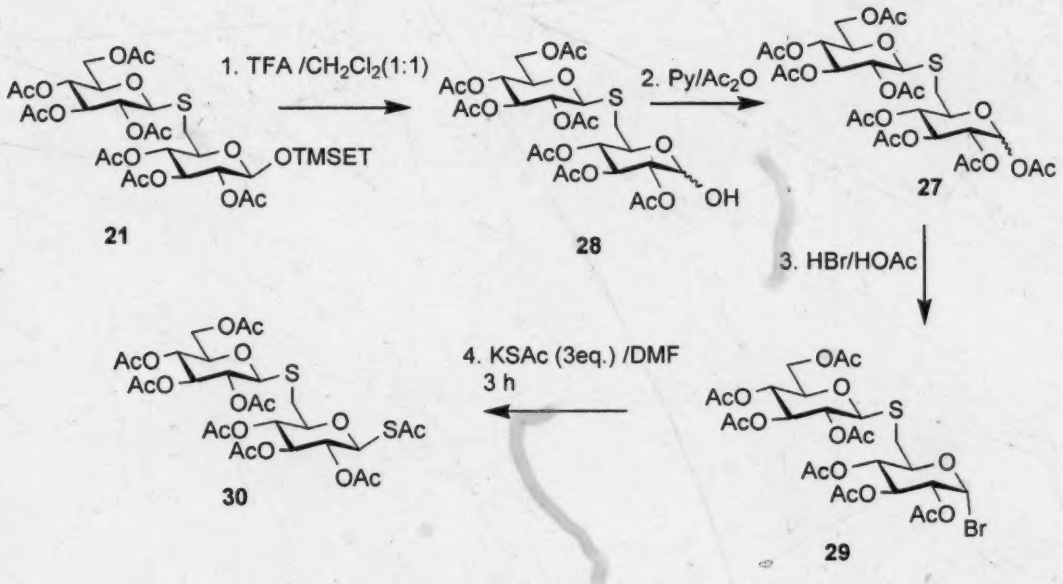
2.4.3 Synthetic Sequence for Conversion of Glycosyl 1-TMSET to 1-SAc

We realized that we could not use an active Lewis acid in our synthetic sequences. Thiooligosaccharides have been known to be more acidic resistant than their *O*-linked counterpart. Thus a protic acid, 50% trifluoroacetic acid (TFA) in CH_2Cl_2 was chosen to convert the TMSET glycoside into disaccharide hemiacetal **28**. The reaction was rapid and specific for the anomeric center. Disaccharide **28** was

obtained in essentially pure form on simple evaporation (**Scheme 2.8**). Repeating additions of toluene during the evaporation process protected the hemiacetal sugar **28** from high concentrations of TFA.



Scheme 2.7 Conversion of TMSET disaccharide **21** to disaccharide 1-*O*-acetate **27.**



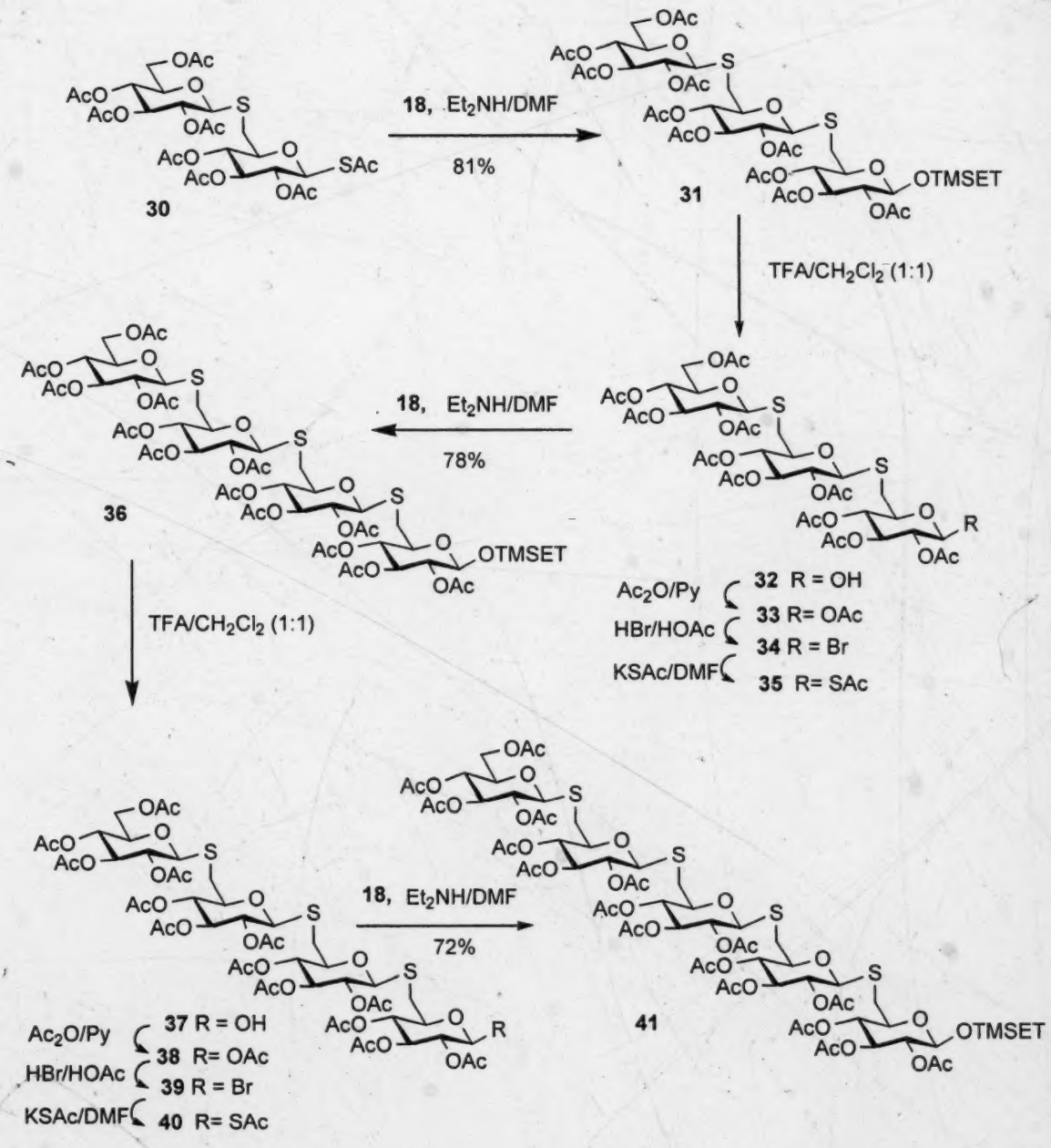
Scheme 2.8 Preparation of disaccharide 1-thioacete **30**.

Acetylation of **28** by acetic anhydride in pyridine gave **27** quantitatively. Bromination was then accomplished with HBr in acetic acid in 85% yield. A simple S_N2 displacement of bromide **29** provided the desired disaccharide thioacetate **30** and the overall yield for the whole process was 71%. Both the de-blocking of the TMSET group and the bromination step indicated that the β-1,6-*S*-linkage survived these harsh acidic conditions. This four-step procedure, from disaccharide **21** to 1-thioacetate **30** became the optimal sequence to achieve the desired transformation as the interglycosidic thio-linkage was unaffected.

2.4.4 Assembly of Linear β-1,6-S-Oligosaccharides

The disaccharide thioacetate **30** was then subjected to the next round coupling with **18**, affording TMSET trisaccharide **31** in 81% yield. The same homologative cycle, made up of the TMSET transformation and sequential *in situ* thioglycosylation, was repeated two more times, generating trisaccharide thioacetate **35** and TMSET tetrasaccharide **36**, along with tetrasaccharide thioacetate **40** and TMSET pentasaccharide **41** respectively (**Scheme 2.9**).

It appeared that we did not reach the limitation of application of the above iterative homologation, and therefore the assembly of a longer thioglycosylation with more than five monosaccharide units, in principle, is feasible.



Scheme 2.9 Assembly of longer β-1,6-S-oligosaccharides **31, 36** and **41.**

2.5 Synthetic Studies Toward Thio-Macrocyclization

2.5.1 Introduction- A Model Study

With linear thiooligosaccharides **31, 36** and **41** in hand, we turned our attention to accomplish the crucial macrocyclization step. Encouraged by the efficient *in situ* thioglycosylation between building block **18** and thioacetates **30, 35** and **40** (**Scheme 2.9**), we considered the introduction of an iodo group and a thioacetate group at the two ends of the linear thiooligosaccharides. We expected such functionalized thiooligosaccharide could undergo intramolecular thioglycosylation to achieve the desired macrocyclization.

In order to test this idea, the simplest cyclic thiosugar, 1,6-*S*-anhyro sugar **42**, was taken as a model molecule to investigate if it could be synthesized from the designed intermediate **43** by an intramolecular displacement reaction.

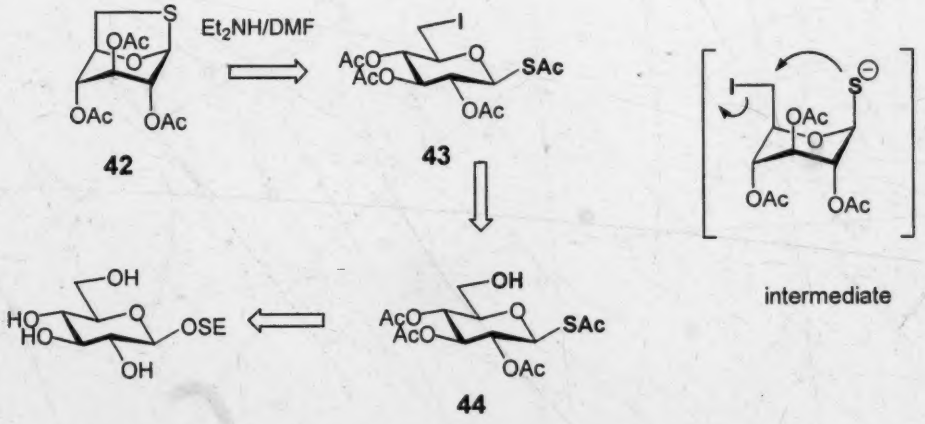
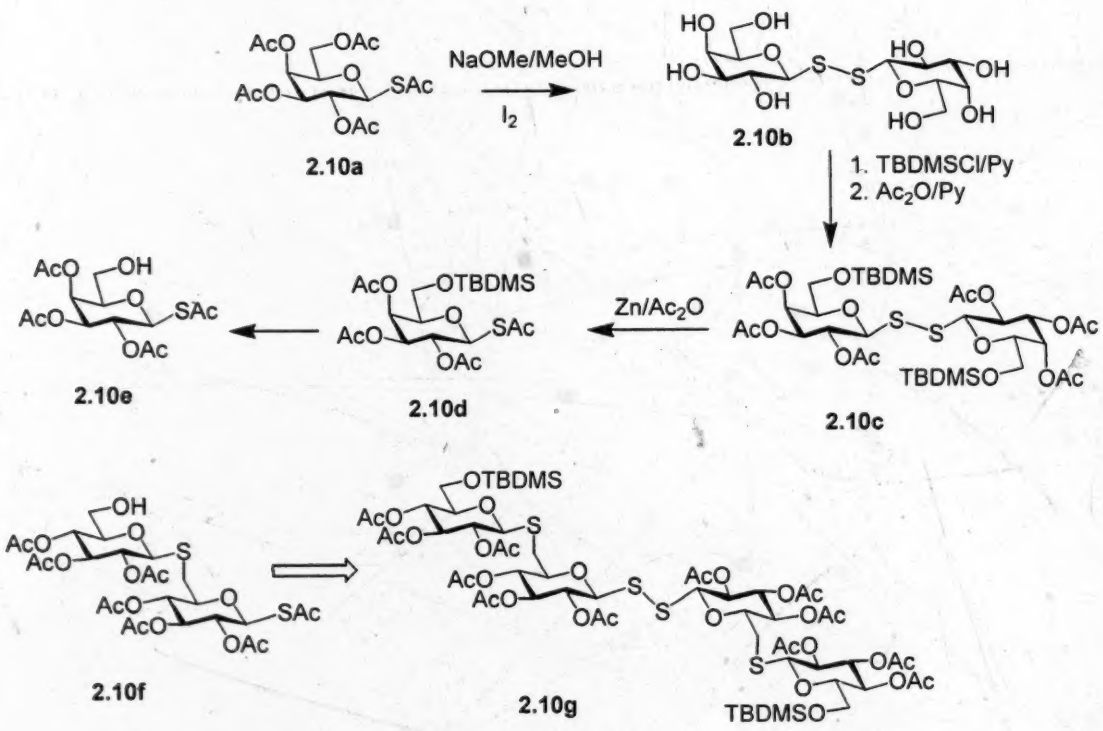


Figure 2.6 Retrosynthetic analysis of model target molecule **42.**

Structure **43** could be derived from the iodination of 2,3,4-tri-*O*-acetyl-1-*S*-acetyl-1-thio-glucopyranose **44.** The key step in this model study was to prepare **44,** ideally from a TMSET glycoside (**Figure 2.6**).

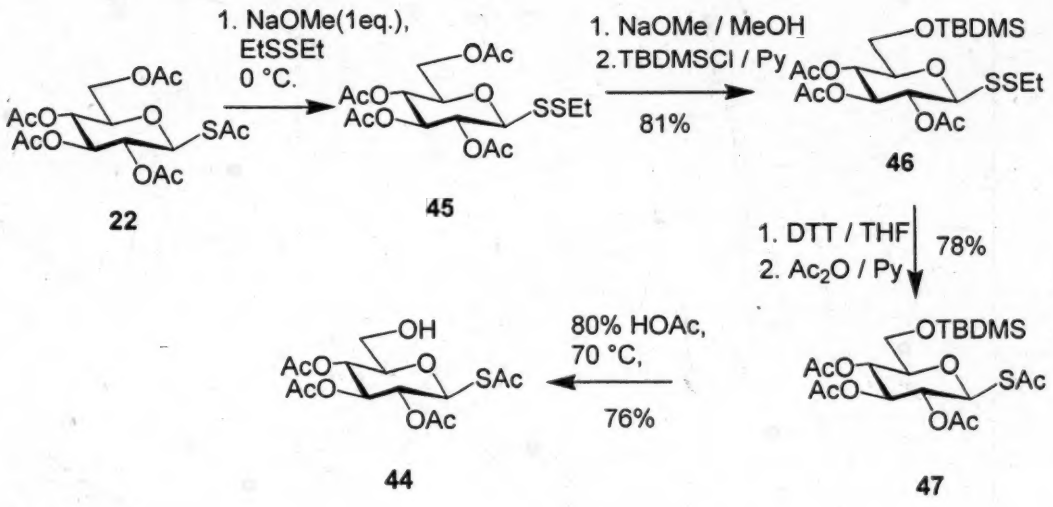
We noticed that the synthesis of compound **43** has never been reported in the literature. There is only example of the synthesis of structure **2.10e,** an analogue of **44,** was reported by Itzstein et al. (**Scheme 2.10**) [150]. In their work, a fully acetated glucosyl thioacetate **2.10a** was first treated with NaOMe affording a thiolate, which was further oxidized into a 1,1-disulfide sugar **2.10b** by the action of iodine. The two primary hydroxyl groups on the disaccharide **2.10d** were then selectively protected as their TBDMS ethers, followed by acetylation to provided **2.10c**. With the TBDMS ether in place, the 1,1-disulfide bond in disaccharide **2.10c** was then reductively cleaved by Zn powder in acetic anhydride, regenerating monosaccharide **2.10c**. Removal of the TBDMS group under acidic conditions gave the final compound **2.10e**. The overall yield of this process was relatively low. Application of this unconventional oxidation-reduction approach would involve the formation of a double-sized linear thiooligosaccharide **2.10c** if applied to our system. This methodology was therefore not practical in our case. For example, in order to prepare disaccharide precursor **2.10f**, the synthetic sequences have to go through a tetrasaccharide intermediate **2.10g**.



Scheme 2.10 Itzstein's synthesis of 2,3,4-tri-*O*-acetyl-1-*S*-acetyl-1-thiol-β-D-galactopyranose **2.10e**.

2.5.2 Disulfide as the Anomeric Protecting Group

Inspired by Itzstein's approach in **Scheme 2.10**, we decided to use a simple ethyl disulfide [151] instead, to protect the 1-thiol with the aim of avoiding the formation of large linear thiooligosaccharides (**Scheme 2.11**).

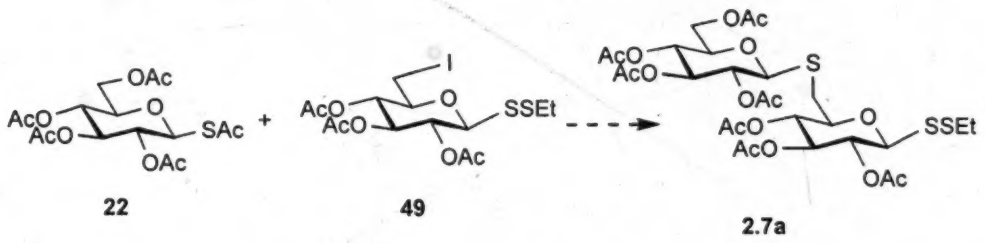


Scheme 2.11 Synthesis of **44** *via* an ethyl disulfide as the anomeric protecting group.

Glucosyl ethyl disulfide **45** was first prepared in moderate yield (61%) by treatment of thioacetate **22** with NaOMe and EtSSEt in THF. Deacetylation of **45**, followed by selectively protecting OH-6 with the TBDMS group and reacetylation gave **46**. The anomeric ethyl disulfide was reductively removed by dithiothreitol (DTT). The resulting glucosyl thiol was reacetylated to give **47**. Subsequent removal of the TBDMS group in **47** afforded the desired structure **44**. This process was efficient and easy to reproduce.

To apply this modified procedure to the preparation of a linear oligosaccharide 1-thioacetate requires the synthesis of a linear thiooligosaccharide disulfide **2.7a** **(Figure 2.7).** A building block **49**, ethyl 2,3,4-tri-*O*-acetyl-6-dexoy-6-iodo-β-D-glucopyranosyl disulfide, was originally designed with the aim of introducing the anomeric ethyl disulfide group at the reducing end of linear thiooligosaccharides. The

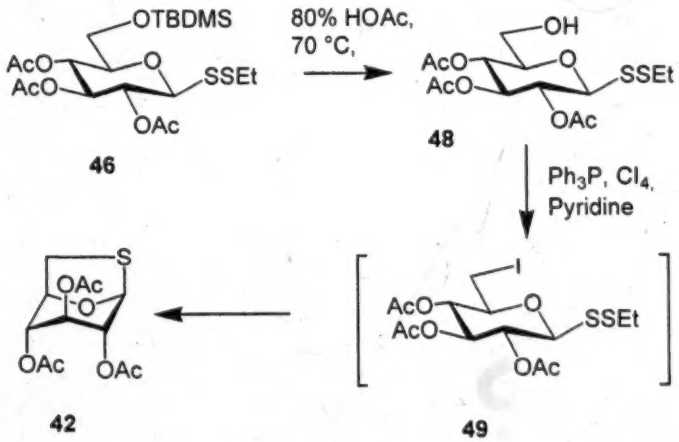
preparation of 1-dithioethyl thiooligosaccharide **2.7a** was expected through an *in situ* thioglycosyl coupling of glycosyl thioacetate with building block **49**.



22 49 2.7a

Figure 2.7 Synthetic route for introducing SSET on a thiooligosaccharide.

The attempted synthesis of building block **49** started with ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosyl disulfide **48**, which was derived from compound **46** by removal of its TBDMS group (**Scheme 2.12**). The subsequent iodination of **48** was expected to give the desired building block **49.** Unfortunately, the proposed building block **49** was extremely unstable and rapidly collapsed into 1,6-*S*-anhydro sugar **42** in the reaction mixture. This unexpected difficulty made us decide to stick with the original precursor (in **2.2b** in **Figure 2.2**) using an acetate as the anomeric protecting group at the reducing end.

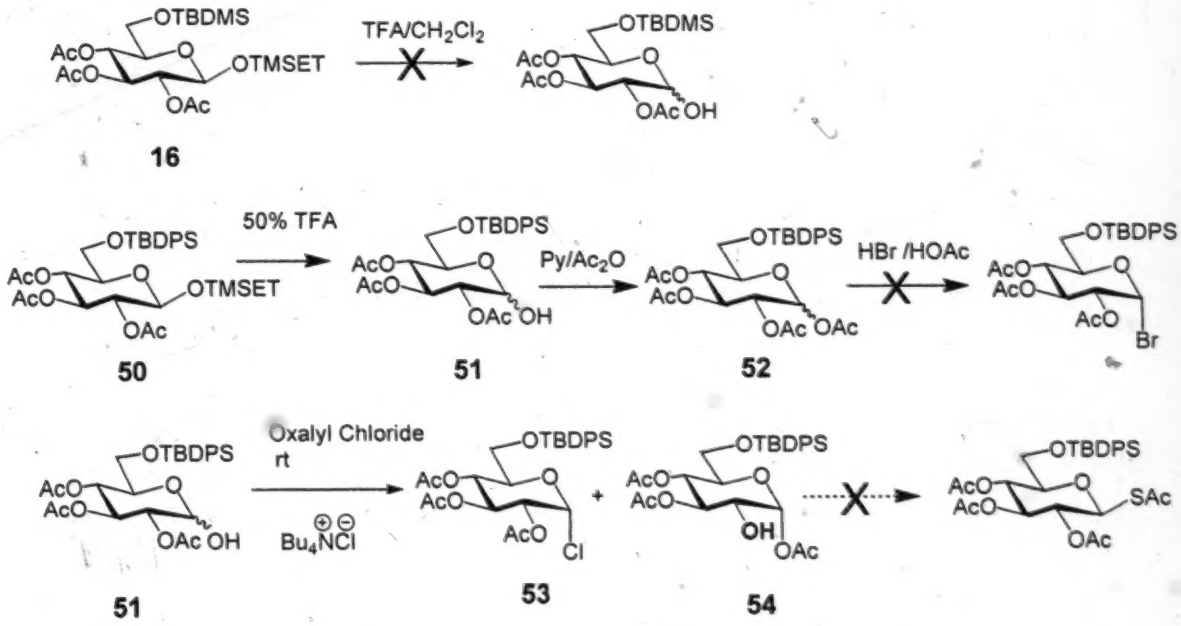


46 48 42 49

Scheme 2.12 An unstable building block **49**.

2.5.3 Protecting Groups for OH-6 at the Non-Reducing Terminus

We decided to go back a few steps by first protecting OH-6 in the TMSET glucoside **17** followed by conversion of the anomeric TMSET into the glycosyl thioacetate (**Scheme 2.13**). We intended to follow the four-step procedure as described in **Scheme 2.8**. The ideal protecting group for OH-6 should withstand those strong acidic conditions. Furthermore, conditions to remove the protecting group have to be mild enough not to affect the base/nucleophile labile 1-thioacetate.



Scheme 2.13 Protecting groups for OH-6 at the non-reducing terminus.

The TBDMS group was first studied and it was found to be cleaved from **16** using 1:1 TFA/CH_2Cl_2. The more acid resistant group *tert*-butyldiphenylsilyl (TBDPS)

was then selected for protecting OH-6. Compound **50** survived 50% TFA and gave hemiacetal **51**. Subsequent acetylation provided the 1-*O*-acetyl derivative **52** quantitatively. Unfortunately, the rest of the process failed as treatment with HBr/HOAc resulted in cleavage of the TBDPS group. Alternatively, the synthesis was attempted from hemiacetal **51**, which was treated with oxalyl chloride with intention to generate glycosyl chloride **53**. But a large amount of side product **54**, derived from hydrolysis of compound **53**, was isolated on workup of the reaction mixture.

Guided by the above investigation, we realized that the best orthogonal group for OH-6 might be a reductively removable group. Only a few examples of such protecting groups are found in carbohydrate chemistry, and one of them is the trichloroethoxy group [152]. The trichloroethoxy group was often employed as an anomeric blocking group and it could be removed by Zn/HOAc (**Figure 2.8**). Introduction of the trichloroethoxy group on non-anomeric positions (e.g.OH-6), however, required strong basic conditions and therefore it is not suitable in our case.

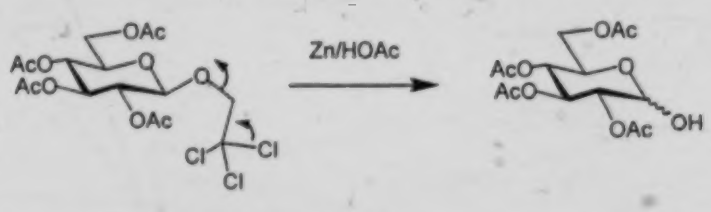


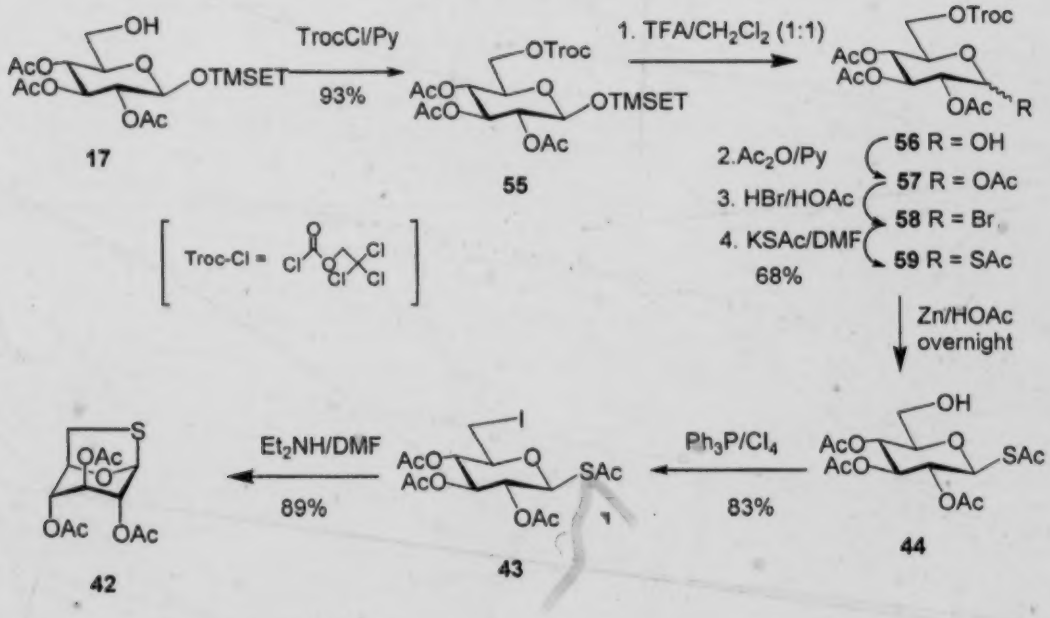
Figure. 2.8 Trichloroethoxy as the anomeric protecting group.

Another candidate, trichloroethoxycarbonyl (Troc), which is most widely employed for protecting amino groups but seldom used for hydroxyl groups [153,154], was then investigated **(Scheme 2.14)**. As an alkoxycarbonyl protecting group, it is

known to be sensitive to basic conditions but should withstand harsh acidic reagents [155-157].

The Troc group was successfully added to OH-6 in compound **17** using its chloroformate at low temperature in the presence of pyridine (**Scheme 2.14**). TMSET glycoside **55** was then transformed into 1-thioacetae **59** by means of the sequential four-step procedure. To our relief, the Troc group was found to be intact in the S_N2 substitution when glycosyl bromide **58** was transformed into the thioacetate **59**. In acetic acid, the Troc group was reductively removed by Zn dust in 72% yield after chromatography. We observed that substantial acetyl migrations occurred from OH-4 to OH-6 under these acid conditions. The OH-6 in **44** was then subjected to the iodination reaction by triphenyl phosphine and tetracarbon iodide in toluene, affording the desired key precursor **43**.

The *in situ* S-deacetylation and intramolecular glycosylation was then achieved on the model compound **43** in 89% yield (**Scheme 2.14**). At this stage, we were confident that this method could be readily applied to the linear thiooligosaccharides to achieve our final goal.

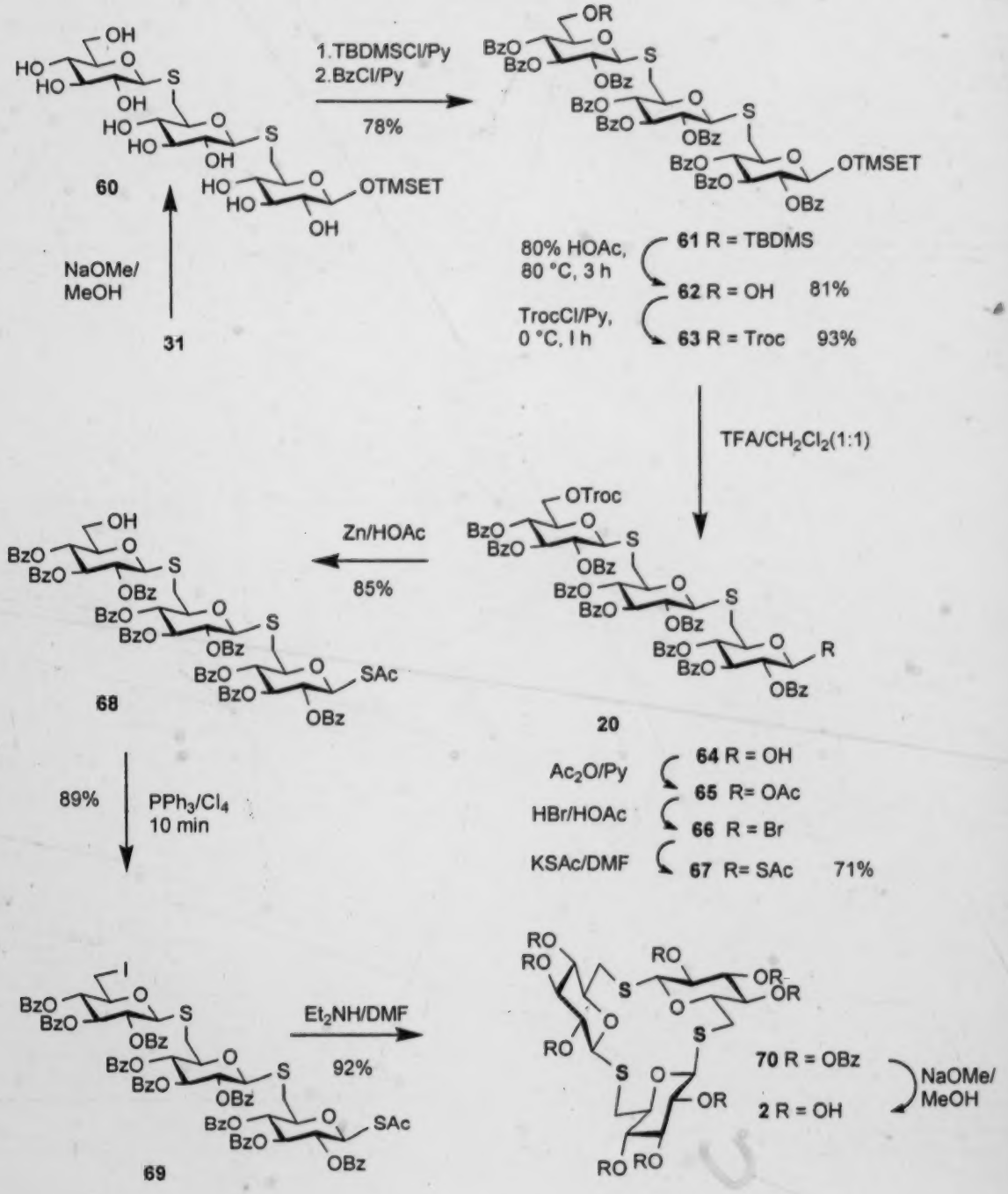


Scheme 2.14 Troc as the protecting group for OH-6.

2.6 Synthesis of Cyclic β-1,6-*S*-linked Oligosaccharides

2.6.1 Synthesis of Cyclic Trisaccharide

To this end, we were in a position to bifunctionalize linear trisaccharide **31** with a 6-iodo and a 1-thioacetate group following the same procedures described in Section 2.5.3. Fully acetylated trisaccharide **31** was first deacetylated to give unprotected linear trisaccharide **60.** The primary OH-group of **60** was protected as its TBDMS ether prior to *O*-benzoylation to give **61** in 78% yield (**Scheme 2.15**). Here, benzoates were chosen to protect the rest of hydroxyl groups, with the intention of avoiding the acetyl migration between OH-4 and OH-6. With benzoates as protecting groups on the linear thiooligosaccharides, the majority of the ring proton resonances of the linear intermediates (**61** to **69**) in ^{1}H 600 MHz NMR spectra were well resolved despite their



Scheme 2.15 Synthesis of cyclic β-1,6-*S*-linked trisaccharide **2**.

repetitive structures as homo-oligomers.

Removal of TBDMS group in **61** by 80% HOAc gave compound **62**. The 6-OH group was then protected as the Troc carbonate group in 90% yield. Attempts to directly install Troc group regioselectively on OH-6 of the unprotected linear trisaccharide **60** was unsuccessful.

With the Troc group installed on 6-position, the anomeric TMSET group in **63** was converted into the trisaccharide thioacetate in four sequential steps without any difficulties, yielding the trisaccharide **67**. Both of the interglycosidic thio-linkages and the Troc group had not been affected by the harsh acidic conditions. The following step was the removal of Troc group in **67** with Zn/HOAc, affording the free primary hydroxyl group. C-6 of **68** was iodinated by using a mixture of triphenyl phosphine, imidazole and iodine at room temperature yielding **69** in 89% yield. (**Scheme 2.15**)

A solution of **69** in DMF (0.25 M) was next treated with Et_2NH at -10 °C. This moderate dilute concentration was used for the macrocyclization step with the intention of avoiding intermolecular reaction. The crucial intramolecular thioglycosylation reaction was achieved, giving benzoylated cyclic thio trisaccharide **70** in surprisingly high yield (92%) (in the contrast to that of its *O*-linked cyclic sugar, which often was below 10%). No α-isomer was isolated from the reaction mixture. Linear oligomers or polymers, which could possibly be derived from intermolecular couplings of precursor **69**, were not detectable by ESI/MS.

The final debenzoylation step under Zemplèn condition provided the fully deprotected cyclic β-1,6-*S*-triasaccharide **2** in almost quantitative yield (**Scheme 2.15**).

2.6.2 Synthesis of Cyclic β-1,6-*S*-Tetrasaccharide and Pentasaccharide

2.6.2.1 Exploration of Polycondensation Approach

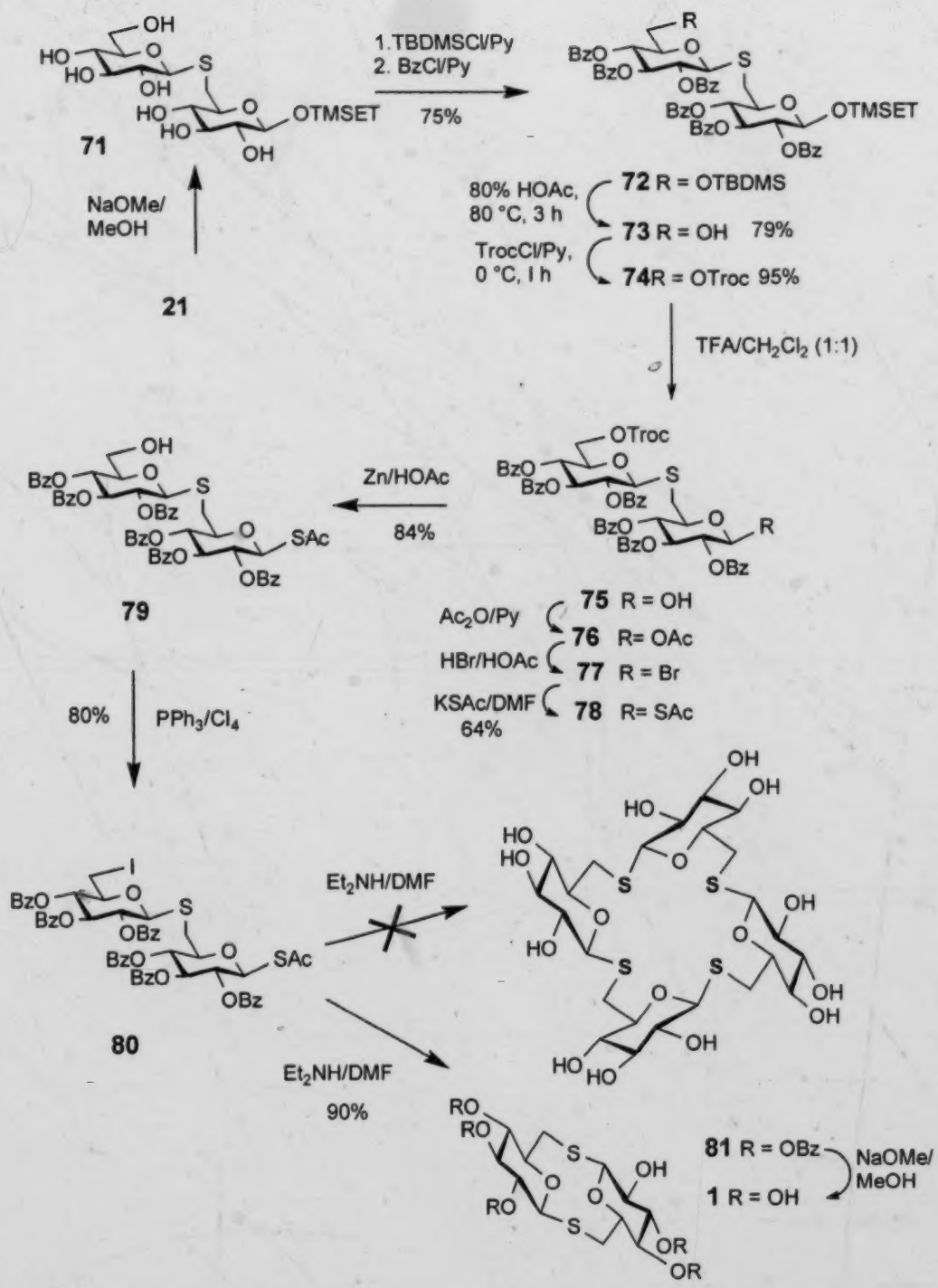
Although cyclic β-1,6-*S*-linked tetrasaccharide could be prepared by means of intramolecular thioglycosylation of the corresponding bifunctionalized linear tetrasaccharide, it would be interesting to explore the possibility of synthesizing it through a polycondensation approach. Ideally, polycondensation of a derivatized repeating unit (a disaccharide monomer), would provide a series of cyclic thio sugars in one pot. Obviously, this approach would be more synthetically efficient to give several novel cyclic thiosugars with different sizes. A disaccharide precursor **80** was thus synthesized (**Scheme 2.16**) from the 1,6-*S*-linked disaccharide **71** following the same methodology used in the synthesis of **70** (**Scheme 2.15**).

A series of solutions of **80** having different concentrations (0.01M, 0.10M and 0.25M) were careful treated with 7 eq. of diethylamine individually at room temperature and were left overnight. Unfortunately we did not separate any cyclic tetramer, hexamer or octamer by chromatography, and only the cyclic disaccharide product **81** was obtained in each case. This suggested that the intramolecular thioglycosylation was the predominant reaction under all these conditions.

Based on the above observations, the larger cyclic β-1,6-*S*-oligosaccharides, cyclic tetrasaccharide **3** and pentasaccharide **4**, had to be synthesized from their corresponding bifunctionalized linear sugars. The total synthesis of cyclic tetrasaccharide and pentasaccharide are described in **Scheme 2.17** and **2.18** respectively, following procedures similar to those in the synthesis of the smaller cyclic thiosugars **1** and **2**.

It is worth mentioning that the crucial macrocyclization step in each case was accomplished in high yield with well-controlled stereochemistry (exclusively anomeric β configuration). The yields were 92% for both cyclic di- and trisaccharide, 95% for tetrasaccharide **3** and 84% for pentasaccharide **4**. Starting from their corresponding unprotected liner thiooligosaccharides, the overall yields for the preparation of cyclic sugars were 22% for cyclic disaccharide **1**, 28% for cyclic trisaccharide **2**, 15% for tetrasaccharide **3** and 12% for pentasaccharide **4**.

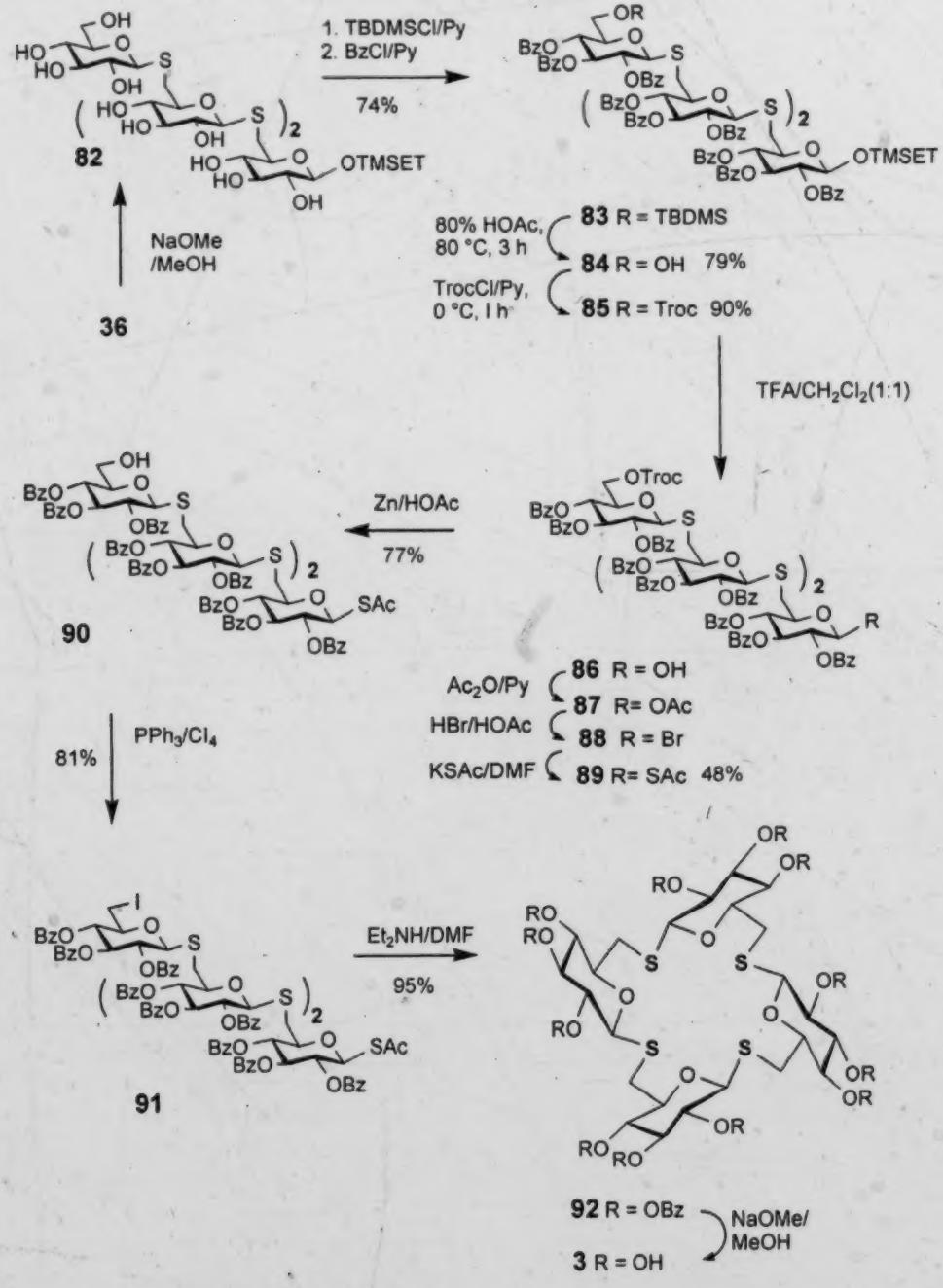
In summary, a methodology for the synthesis of β-1,6-*S*-linked cyclic glucopyranosides was successfully developed, and the preparation of these novel cyclic sugars provides us with opportunity to investigate their unique physical properties. This methodology can be potentially extended to synthesize other cyclic thiosugars with different linkages (will be discussed in Chapter 4).



Scheme 2.16 Attempted polycondensation of a disaccharide unit **80.**

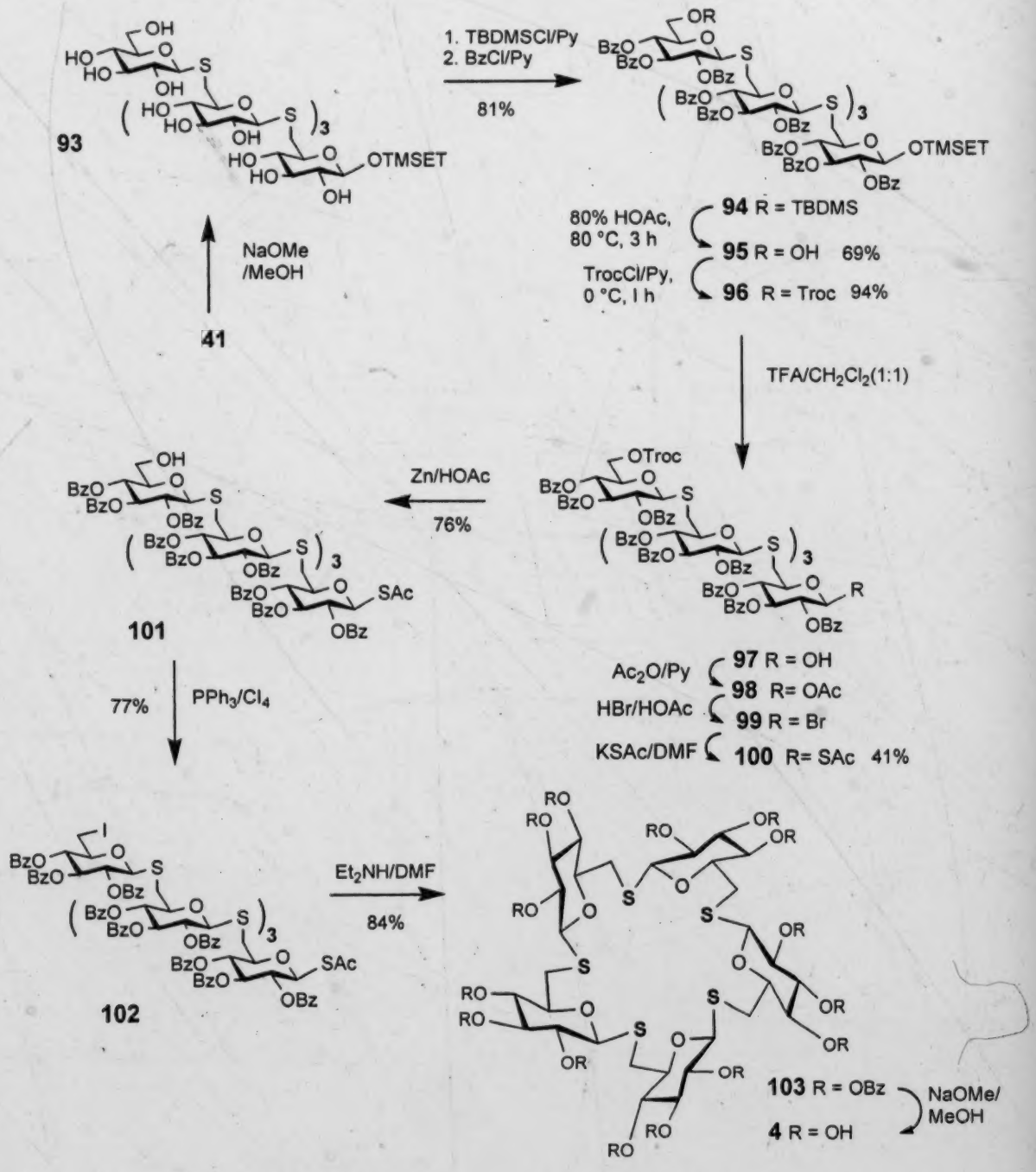
2.6.2.2 Synthesis of Cyclic β-1,6-*S*-Tetrasaccharide and Pentasaccharide

Cycle A: Synthesis of β-1,6-*S*-Linked Cyclic Tetrasaccharide 3



Scheme 2.17 Synthesis of cyclic *S*-linked tetrasaccharide **3**.

Cycle B: Synthesis of β-1,6-*S*-Linked Cyclic Pentasaccharide 4:



Scheme 2.18 Synthesis of cyclic *S*-linked pentasaccharide **4.**

2.7 Structural Characterization of Cyclic β-1,6-*S*-Linked Oligosaccharides

In the course of synthesizing cyclic thiooligosaccharides, a series of linear oligosaccharide intermediates were prepared and their ^{1}H NMR spectra were recorded. Surprisingly, all the spectra displayed well-dispersed proton signals although all these linear oligosaccharide intermediates are homo-oligomers composed of only glucopyranoside units. For example, 21 protons on the three glucopyranoside rings of benzoylated trisaccharide precursor **69** were unambiguously assigned by two-dimensional ^{1}H-^{1}H correlation spectroscopy (COSY) NMR (**Figure 2.9**).

The ring proton resonances in the longer linear oligosaccharides, tetra- and pentasaccharides, were also evenly distributed without severe overlap. Although the proton signals from the middle pyranoside rings of these linear oligosaccharides can not be easily assigned by 2D COSY NMR, full assignments can be achieved by means of total correlation spectroscopy (TOCSY) NMR technique. The glucose units are therefore all slightly different from each other.

2.7.1 NMR Spectrum of Cyclic Disaccharide

The ^{1}H NMR spectra of benzoylated cyclic disaccharide **81** in different solvents ($CDCl_3$, CD_2Cl_2, benzene-d_6, acetone-d_6 and DMSO-d_6) exhibited unusual proton signals (**Figure 2.10**). In the standard ring proton resonance region (6.0-2.5 ppm), several broad peaks were observed along with a sharp triplet at about 5.9 ppm. The benzoate groups gave normal sharp signals in aromatic proton shift range of 7.0 – 8.0 ppm.

It was thought that this highly rigid molecule might have several conformational isomers, which were slowly interconverting into each other at room temperature. The flat-topped peaks resulted from coalescence of the proton signals from the conformational isomers [158]. Population-averaged signals would be expected when the spectrum is recorded above the coalescence temperature (the coalescence temperature is near the ambient probe temperature, 27 °C in this case).

After removing the benzoate protecting groups in **81**, the resulting free cyclic disaccharide **1** was subjected to ^{1}H NMR in DMSO-d_6 at different temperatures (rt, 45 °C, 70 °C and 100 °C). The ^{1}H NMR spectrum of cyclic sugar **1** was finally well resolved at 100 °C with correct signal multiplicities, and the assignment of all the protons was possible using a 2D NMR experiment. As shown in Figure **2.11**, the spectrum displayed only one set of proton signals. This indicated the structure of the two glucopyranoside rings was symmetrically related with each other. The signals for H-2 and H-3 were severely overlapped, resulting in a higher order signal for H-1 that is normally a simple doublet.

2.7.2 NMR Spectra of Cyclic Trisaccharide, Tetrasaccharide and Pentasaccharide

^{1}H NMR spectra of larger-size cyclic sugars **2**, **3** and **4** were recorded at room temperature, and one simple set of correlated proton signals corresponding to the monomeric unit of the cycloglucopyranosides was observed in each case. All of the repeating units in these cyclic sugars are equivalent on NMR scale. This proved that the structures of all these sugars are completely symmetrical. (**Figure 2.12**)

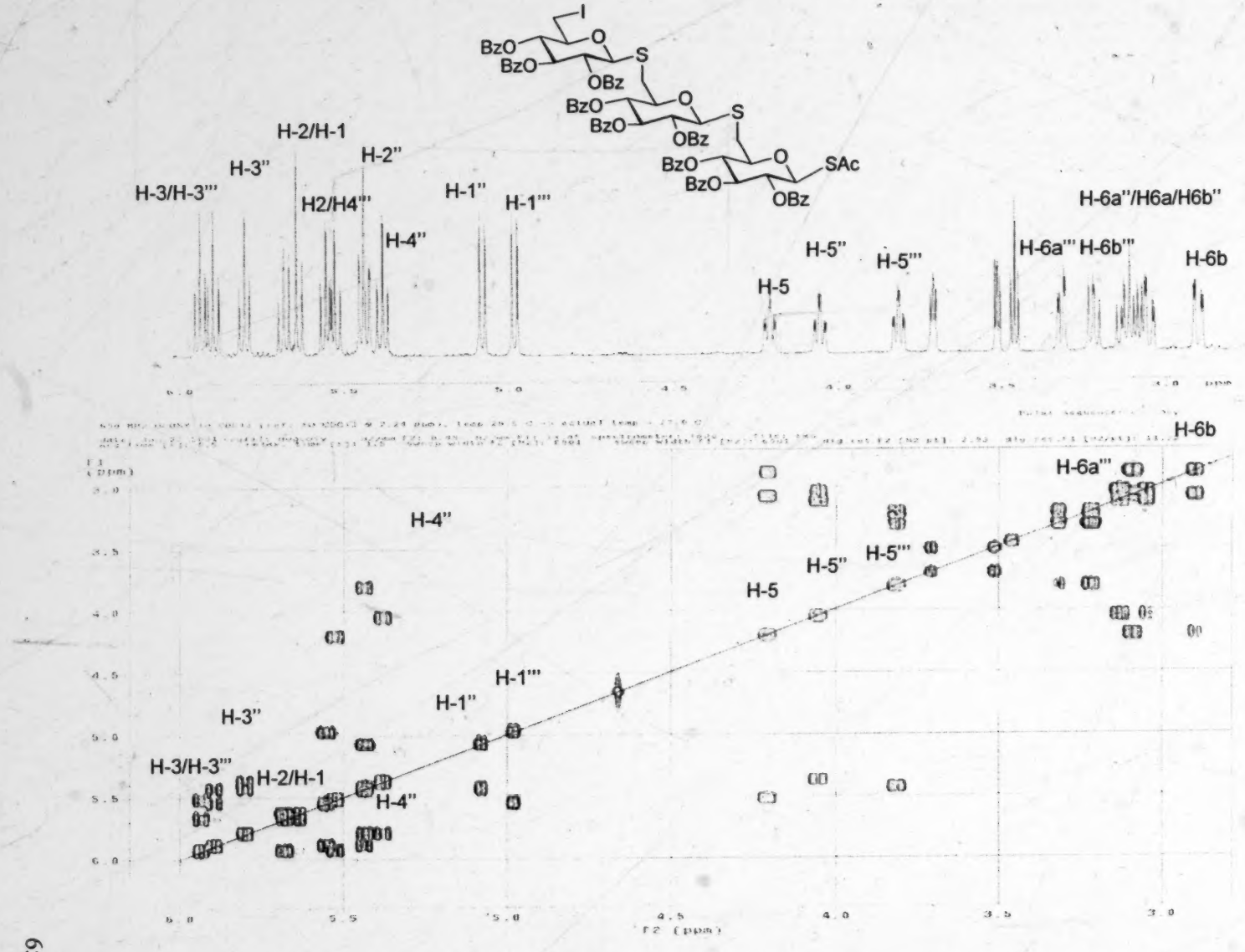


Figure 2.9 ^{1}H 1D and 2D COSY NMR spectra of trisaccharide linear precursor **69** in $CDCl_3$

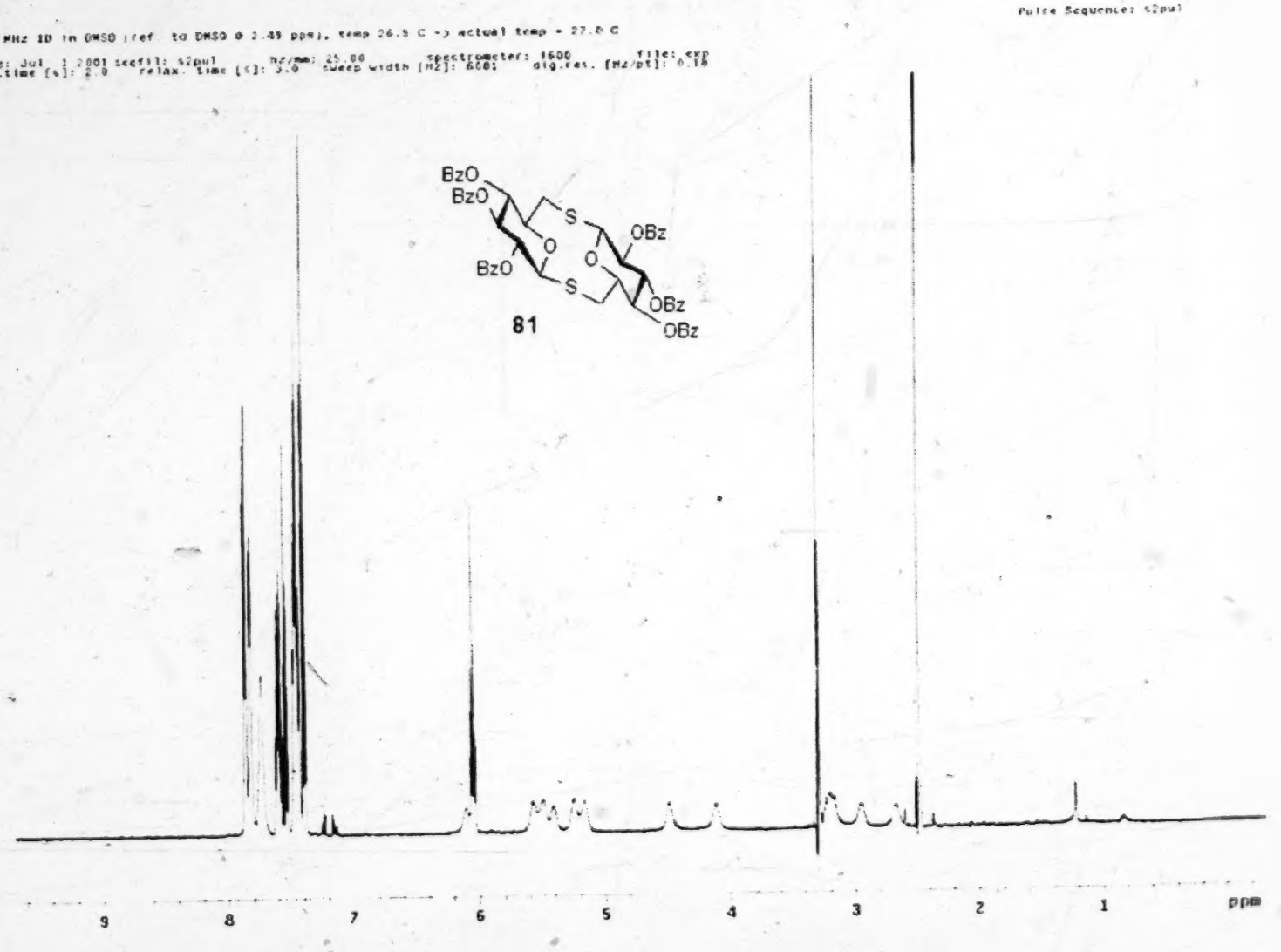


Figure 2.10 ^{1}H NMR spectrum of benzoylated cyclic disaccharide **81** in DMSO-d_6 at rt

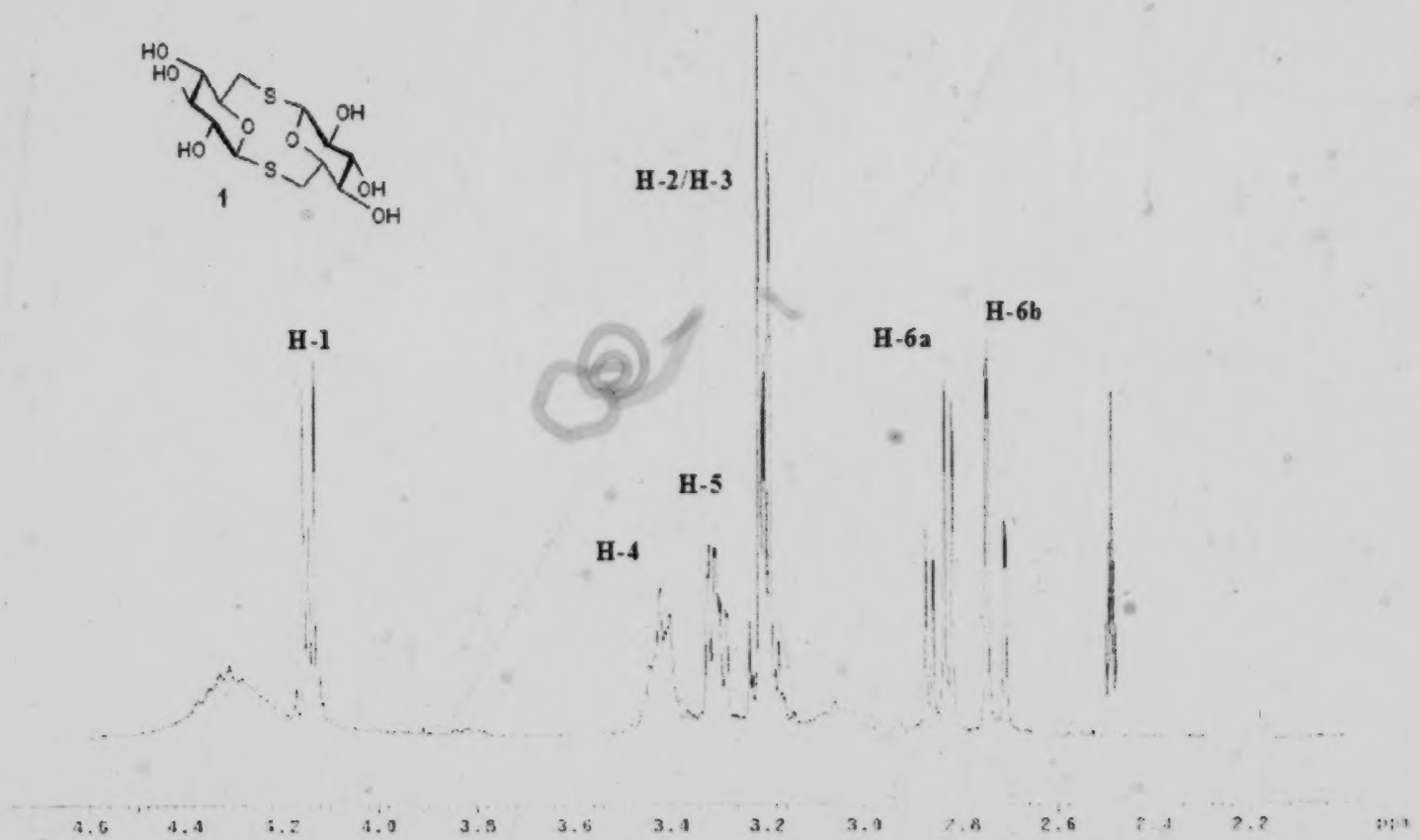


Figure 2.11 ^{1}H NMR spectrum of free cyclic disaccharide **1** in DMSO-d_6 at 100 °C

From cyclic trisaccharide **2** to pentasaccharide **4**, the spectra presented a similar pattern in terms of chemical shift and coupling constant. For all the cyclic thiosugars, the magnitudes of their vicinal H-H coupling constants were characteristics of a repeating glucopyranose ring having a $^4C_1$ chair conformation ($J_{1,2}$ = 10.0 Hz, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.0 Hz, $J_{5,6a}$ = 15.0 Hz, $J_{5,6b}$ = 8.0 Hz). The magnitude of the coupling constants $J_{1,2}$ (10 Hz) confirmed the presence of only β anomeric configurations.

It was interesting to note some trends in these cyclic sugars as their ring sizes increased **(Figure. 2.12)**. From cyclic trisaccharide **2**, tetrasaccharide **3** to pentasaccharide **4**:

(1) The resonances of H-2/H-4 tended to get more overlapped with each other. The merging of C-2/C-4 was also observed in their ^{13}C NMR spectra.

(2) H-6a shifted downfield from 3.09 ppm in trisaccharide **2**, and to 3.26 ppm in pentasaccharide **4**. This implied that as the ring size increased, H-6a moved away from its neighboring sulfur atom and became less shielded from sulfur atom.

(3) In the cyclic trisaccharide spectrum, one of the H-6's was a broad doublet. From cyclic tetrasaccharide to pentasaccharide, this doublet turned into a sharper doublet of doublets with coupling constants increasing from 1.7 to 2.8 Hz.

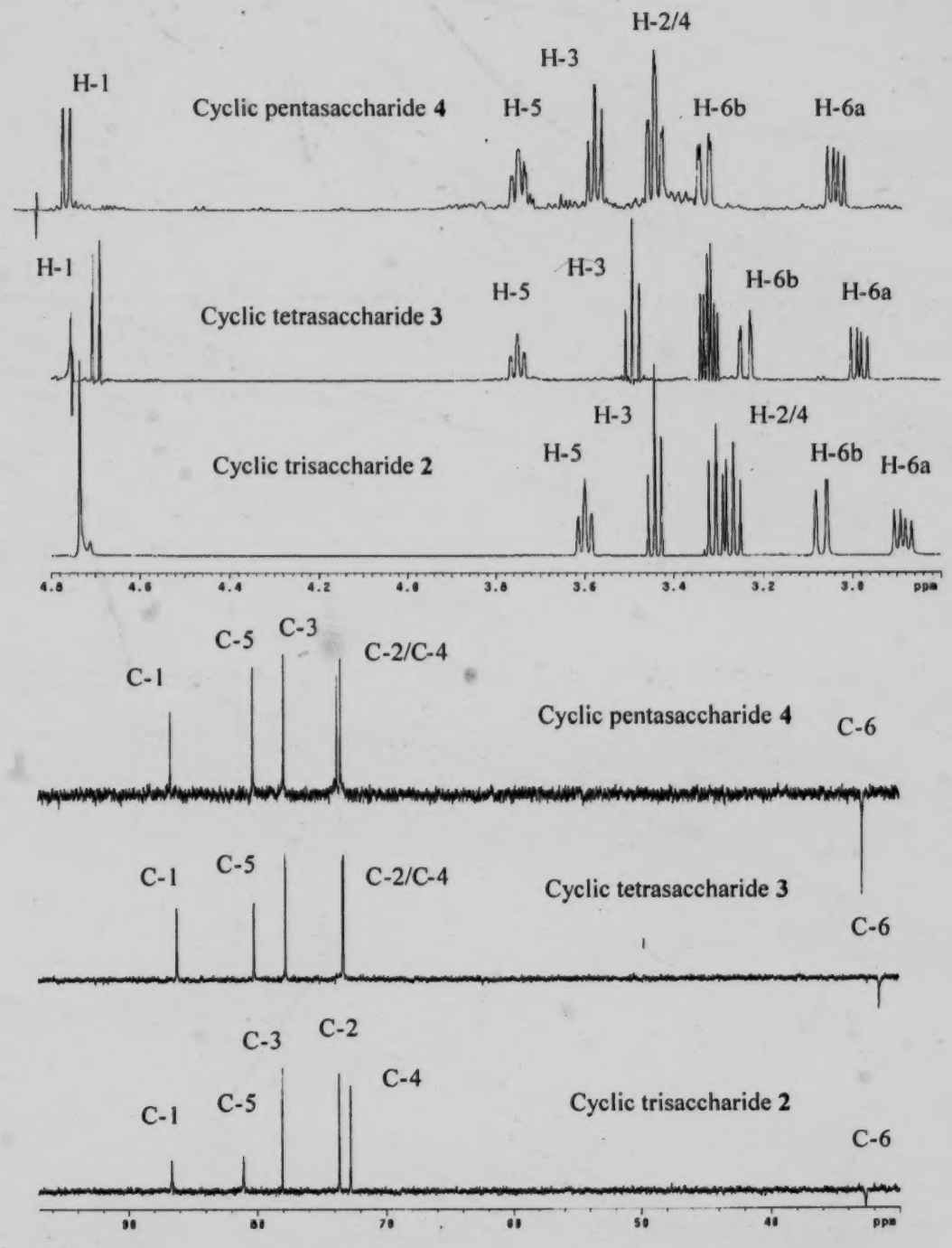


Figure 2.12 ^{1}H and ^{13}C NMR spectra of cyclic tri- and tetra- and pentasaccharides.

(In the ^{1}H NMR spectrum of **2**, H-1 is partially saturated due to HOD suppression)

2.7.3 3D Structures of Cyclic β-1,6-S-Linked Oligosaccharide 1, 2, 3 and 4

In a 1,6-linked oligosaccharide, glycosidic junction through the 1,6-linkage between two residues **a** and **b** offers three contiguous variable torsion angles: Φ, Ψ and ω as shown in **Fig. 2.13.** Three-dimensional structures of these oligosaccharides are primarily determined by these torsion angles. The preferred conformations about these linkages can in principle be determined from energetic calculations together with experimental nuclear overhauser effect (NOE) and spin coupling constants.

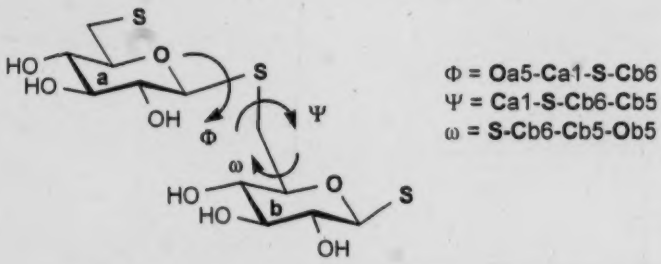


Figure 2.13 Three torsion angles of a 1,6-linkage.

Nuclear overhauser effect spectroscopy (NOSEY) is a powerful NMR technique for conformational analysis in solution. In two-dimensional NOE experiments, the integrated intensity of the cross-peaks between two protons can be directly correlated with the internuclear distance *r* of the observed protons *via* the known r^{-6} dependence. The distance between two H-6 protons is usually chosen as the internal distance calibration. The use of rotating frame overhauser spectroscopy (ROSEY) allows quantification of H-H distance up to approximately 4 Å. However, unlike proteins and nucleic acids, oligosaccharides rarely contain enough inter-residue NOE contacts to define their conformations unambiguously, especially in the case of 1,6-linkages. Furthermore, NOE data in oligosaccharides may not provide sufficient constraints when used alone. The availability of a full molecular mechanical force field parameterized for

oligosaccharides is essential. In our case, the NMR spectra of cyclic *S*-1,6-linked oligosaccharides exhibited only one set of ring proton signals, which could be viewed as a total overlapping of the proton resonances from each individual glucopyranoside unit. Thus, it is difficult for ROSEY experiment to reveal all the possible NOE contacts that may exist between two different residues in the cyclic sugars.

Molecular modeling of these cyclic thiooligosaccharides is also difficult to perform because the parameterization of thioglycosidic linkage in most available force fields is not available. Thioglycosidic linkages have a weaker anomeric effect than the corresponding *O*-glycosides [159]. No parameterization has been developed so far to describe this energy constraint. Although a limited number of conformational studies have been carried out on complex thioglycoside [160-165], the attempted modeling did not cover exo-anomeric effect.

Due to lack of sufficient NOE data and the parameters required for molecular modeling, we decided to use Insight II, a 3D graphical windowed interfere program from *Biosym Technologies*, to construct models of these cyclic thiooligosaccharides at this preliminary stage. The energy minimization process was carried out well with cyclic disaccharide **1** and trisaccharide **2**; although we experienced difficulties with cyclic tetra- and pentasaccharides, **3** and **4**. Both stick and space-filling models obtained for each cyclic sugar are illustrated in **Figure 2.14** to **Figure 2.17,** not to indicate the preferred conformations but simple to help visualize these cyclic compounds.

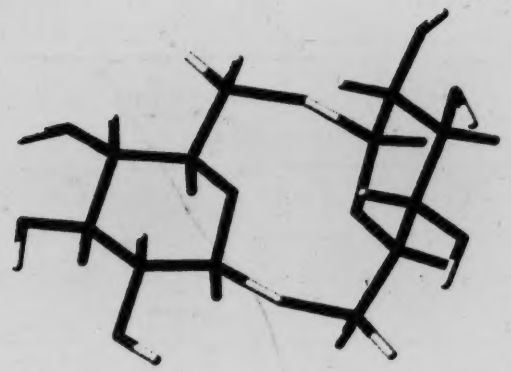
The space-filling models of these cyclic thiosugars revealed that they exhibit torus-like shapes. The maximum molecular size for each cyclic *S*-1,6-linked oligosaccharide is about 10.8 Å for trisaccharide **2**, 11.2 Å for tetrasaccharide **3** and 12.5 Å for pentasaccharide **4**. All of them are about 3.5 Å in width. The glycosidic sulfur atoms are located at the bottom of the cavities. In the cyclic trisaccharide **2**, sulfur atoms are surrounded by hydrogen atoms while in cyclic tetra and pentasaccharide, their sulfur atoms are pointing more outward.

The C1-S-C6 bond angles, the radii of the polygon containing two to five sulfur atoms and the sizes of the cavities are summarized in **Table 2.1**

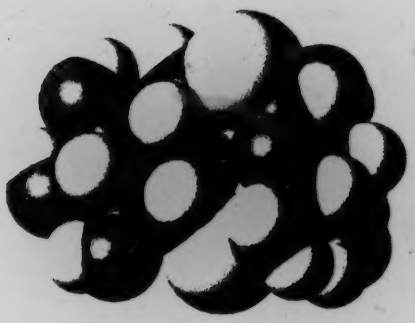
Table 2.1 Bond angles, radii of polygon and sizes of the cavities of cyclic thiosugars

	Bond angle (C1–S–C6)	Radii of polygon	Size of cavities [166] (Radii)
Disaccharide **1**	175.38°	1.30 Å	0.54 Å
Trisaccharide **2**	139.74°	1.90 Å	1.14 Å
Tetrasaccharide **3**	133.99°	4.30 Å	3.53 Å
Pentasaccharide **4**	128.64°	4.97 Å	4.20 Å

Stick Model



Space-filling model
(Back view)



Space-filling model
(Side view)

Space-filling model
(Back view)

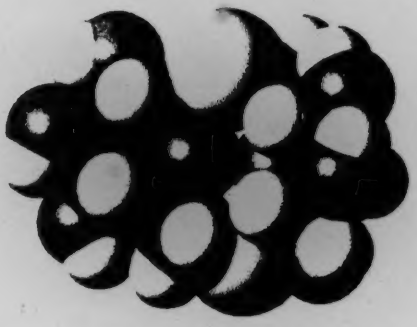


Figure 2.14 Modeled 3D structure of cyclic disaccharide **1**

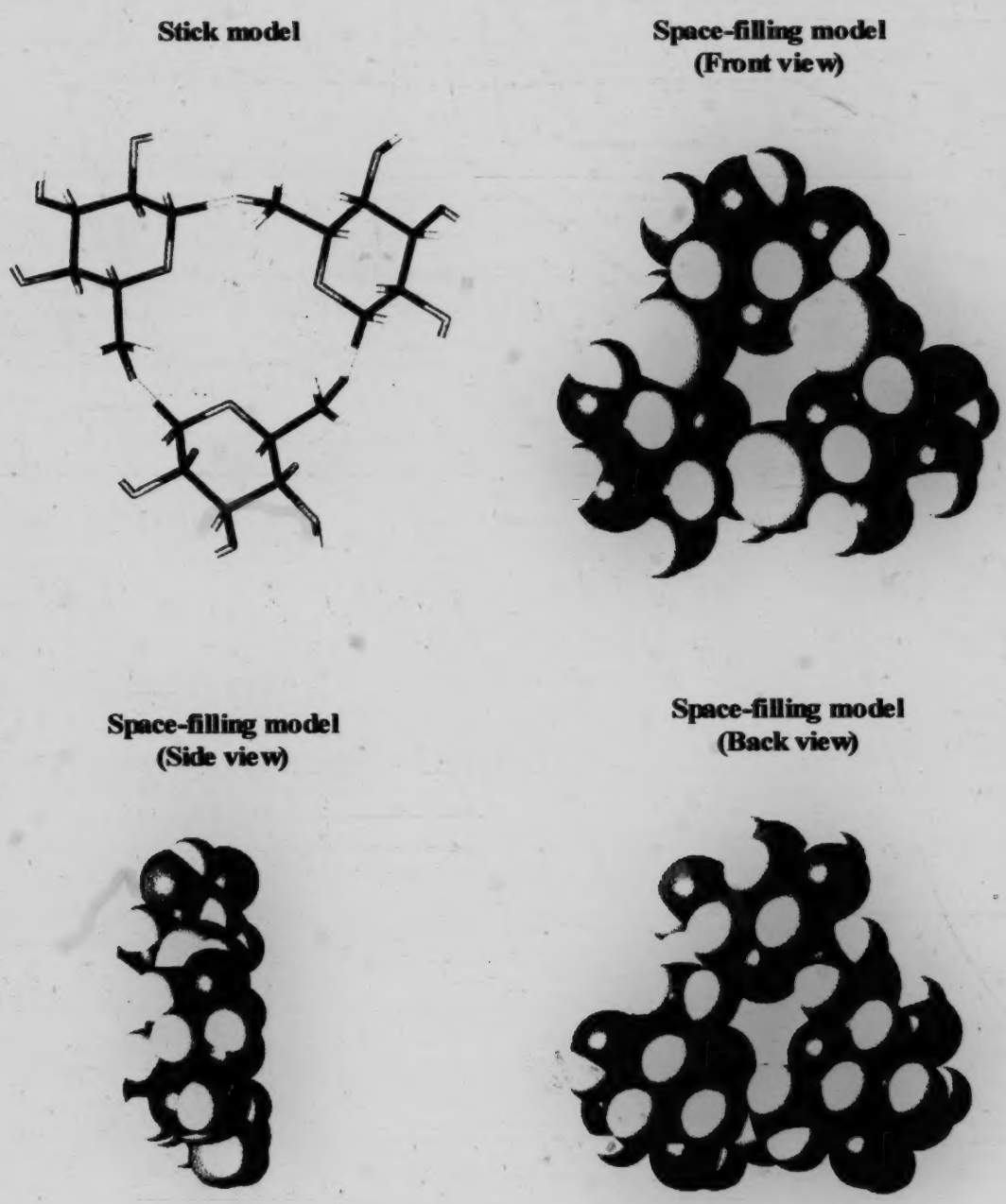


Figure 2.15 Modeled 3D structure of cyclic trisaccharide **2**

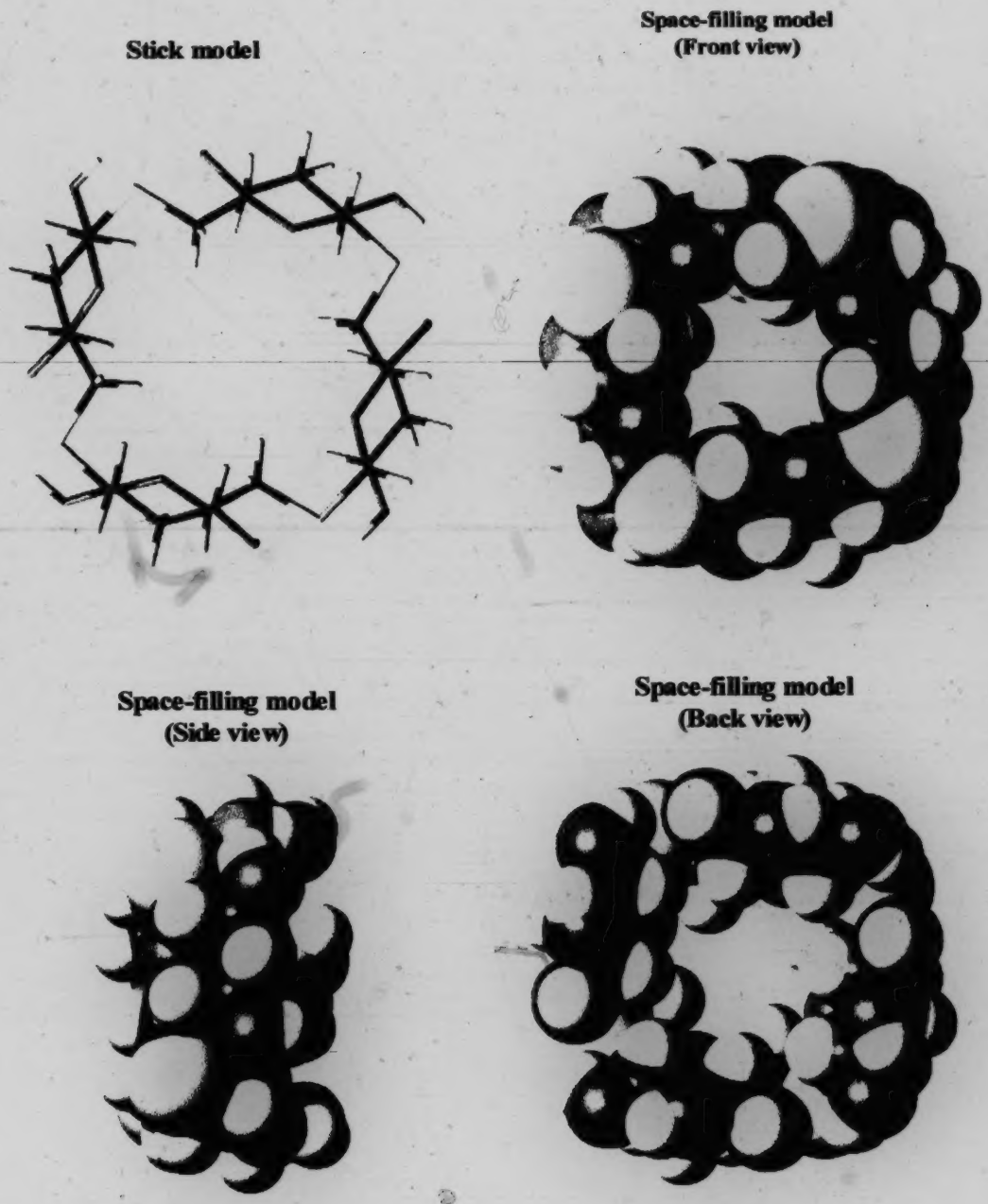


Figure 2.16 Modeled 3D structure of cyclic tetrasaccharide **3**

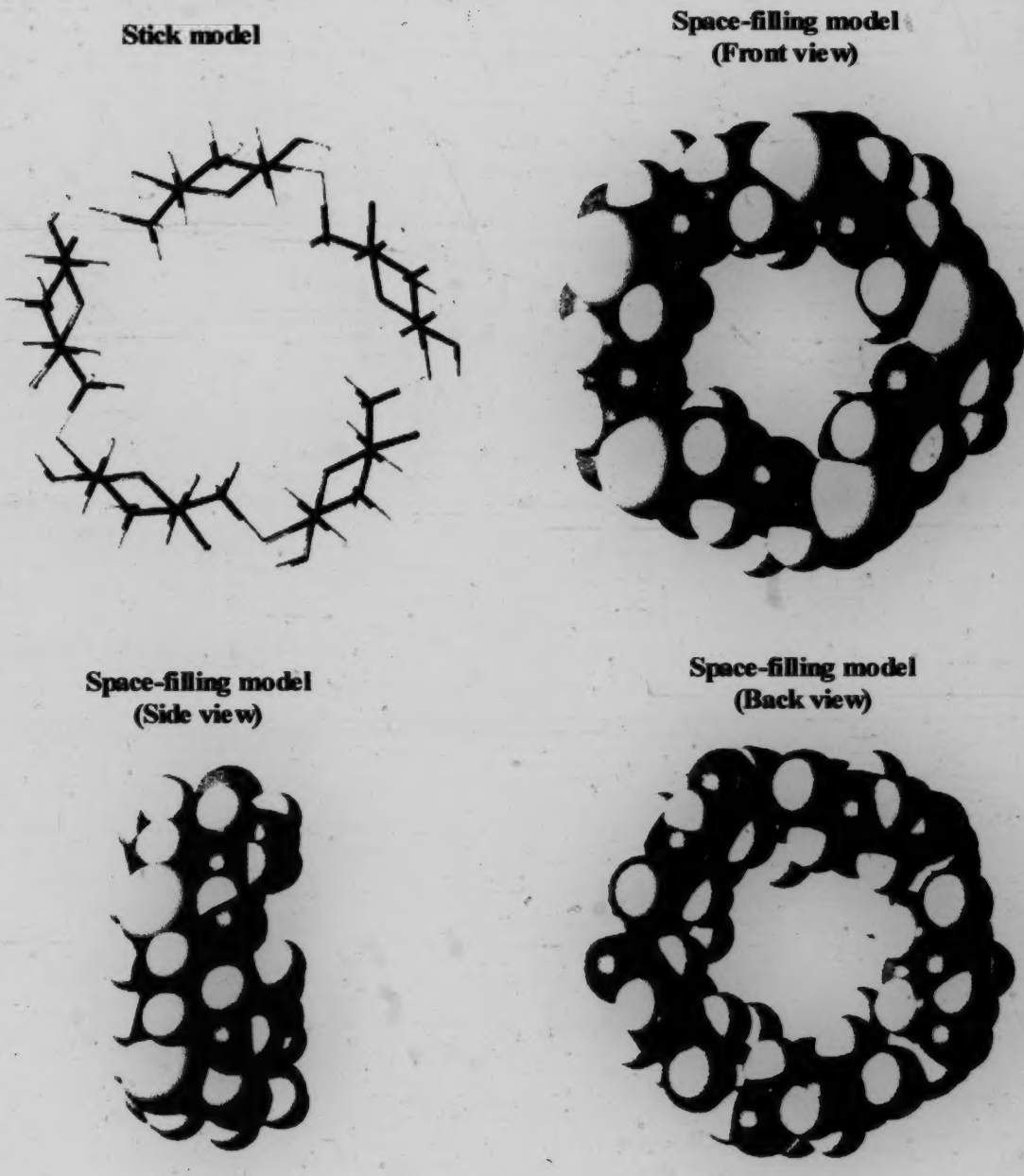


Figure 2.17 Modeled 3D structure of cyclic pentasaccharide **4**

Chapter 3

Preliminary Study of Metal-Ion Complexation With Cyclic β-1,6-*S*-Linked Oligosaccharides

3.1 Introduction

Heavy metals are one of the most hazardous classes of environmental pollutants [167-169]. They can cause serious health problems due to their non-biodegradability. Novel macrocycles have been developed and evaluated as heavy metal extraction agents [170-173]. Recently there has been an increasing interest in the use of sulfur-containing cyclic compounds for the removal of heavy metals *in vivo*. This is due to the fact that sulfur atoms in macrocycles can bind to transition-metal ions effectively through polydentate coordination. This chapter describes our preliminary metal-ion binding study on the carbohydrate-based thio-macrocycles. The complexation of silver ion with cyclic β-1,6-*S*-linked oligosaccharides **2**, **3** and **4** is demonstrated.

3.2 Structural Features of Cyclic β-1,6-*S*-Linked Oligosaccharides

The structures of cyclic β-1,6-*S*-linked oligosaccharides **2**, **3**, and **4** are essentially those of *S*, *O* mixed crown ethers bearing different numbers of glucopyranoside rings **(Figure 3.1)**. The macrocyclic components of cyclic thiooligosaccharides are composed of repeating one and two carbons alternating between ring oxygens and anomeric sulfurs, conferring both acetal and ether-like functionalities upon the macrocycles. The

remaining portions of the pyranose rings occupy the outer surface of these cyclic oligosaccharides.

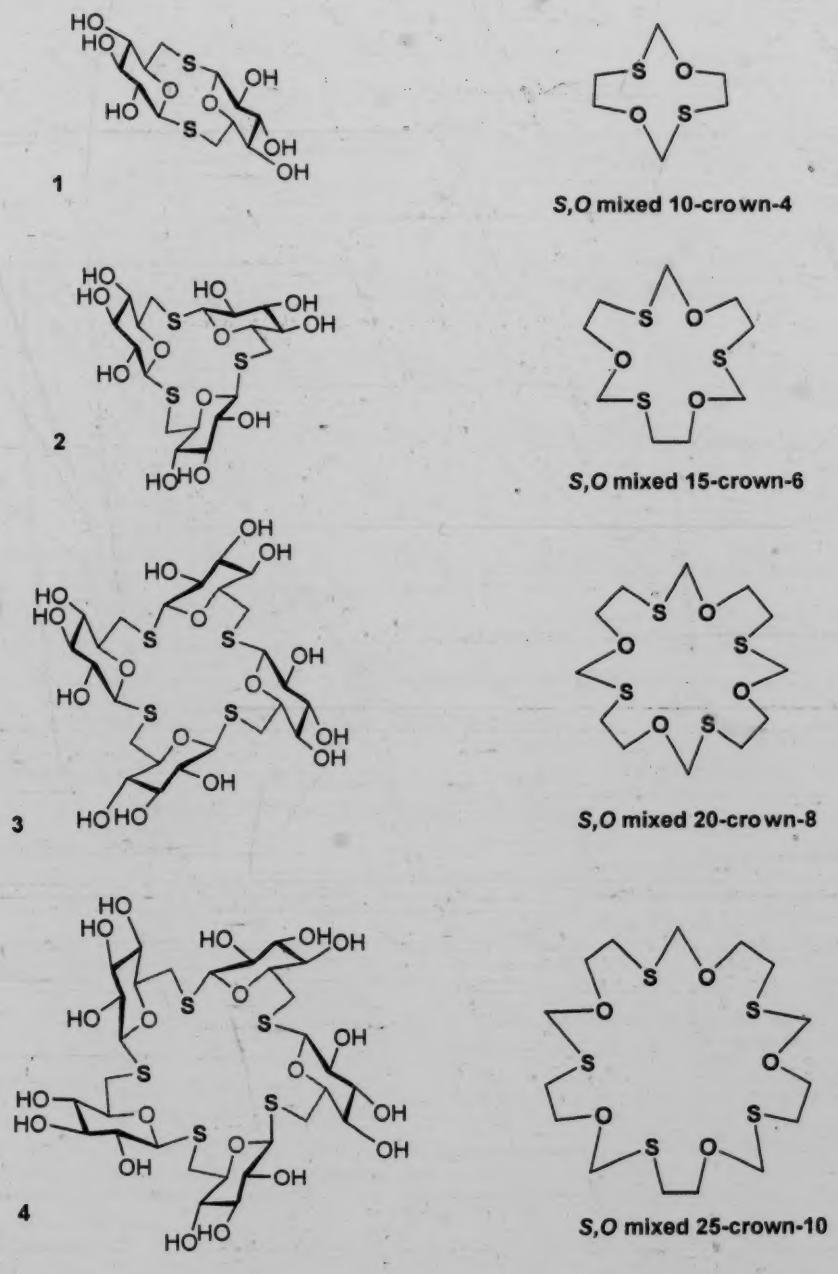


Figure 3.1 Schematic representation of the core structural resemblance of cyclic β-1,6-*S* linked oligosaccharides to *S,O*-mixed crown ethers.

The ring sizes of these carbohydrate macrocycles vary from 10-membered disaccharide, through 15-membered trisaccharide and 20-membered tetrasaccharide, up to 25-membered pentasaccharide with 10 mixed donor atoms (S and O).

Compared to conventional crown ether compounds, these carbohydrate-based macrocycles present some unique structural features. Firstly, they are chiral macrocycles bearing many stereogenic centers originating from glucopyranose units. Secondly, they are water-soluble. Thirdly, the hydroxyl groups on these macrocycles can be readily derivatized with many kinds of functional groups. Fourthly, as analogues of thiacrown ethers, these macrocycles should be capable of binding many transition-metal ions, especially heavy metal ions. It is worth to mention that the hydroxyl groups on glucopyranose units may also compete in coordination with metal ions.

3.3 Screening of the Complexation of Metal Ions with Cyclic β-1,6-*S*-Linked Oligosaccharides by ESI-MS

Conventional methods for the study of metal binding properties, such as potentiometry, spectrophotometry and NMR titrimetry [174,175], have been the most popular methods but these techniques often suffer from low sensitivity and limited versatility for many applications. In recent years, electrospray ionization mass Spectrometry (ESI-MS) has emerged as a new method for host-guest complexing study [176-179]. A variety of coordination compounds have been analyzed by ESI-MS, including transition-metal complexes of biological molecules such as peptides, proteins, carbohydrates, etc [180]. In ESI-MS, the complexes are directly ionized and transferred

gas phase from solution while maintaining the complexes intact under well-controlled mild ionization conditions. The very mild ionization conditions makes ESI-MS the method of choice for the characterization of metal complexes, especially when kinetically labile, paramagnetic metal complexes are involved.

In this study, the metal ions that were chosen to test against three individual cyclic β-1,6-*S*-glucopyranosides by ESI-MS experiments included:

- Alkali metal (Li^+, Na^+, K^+, and Cs^+) and alkaline earth metal ions (Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}).
- Transition-metal ions (Cr^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^+, Cu^{2+}, Zn^{2+}, Mo^{2+}, Ru^{3+}, Pd^{2+}, Pb^{2+}, Ag^+, Cd^{2+}, Au^+, Au^{3+}, and Hg^{2+}).
- Lanthanum (La^{3+}).

Metal nitrate or chloride solutions at a concentration of 1.0 mM were prepared in deionized water. Each metal ion solution was mixed with one of three individual cyclic sugars **2, 3** and **4** solutions with average concentrations of 0.2 mM. The molar ratio of metal-ion to cyclic sugar was controlled at around 5:1. It was expected that a larger concentration of metal ions would promote a faster equilibration. The equilibrated solutions were then subjected to ESI-MS analysis.

For all the alkali and alkaline earth metal ions, the three cyclic sugars gave intensive ESI-MS peaks corresponding to the complexes (molecular weight plus metal ion). No complexation was observed for La^{3+}. The heavy metal ions Pb^{2+} and Hg^{2+} did not show any complexation ions either. This observation is in contrast to the strong binding of heavy metal ions with normal thiacrown ethers. Other transition-metal ions,

such as Mn^{2+}, Mo^{2+}, Ru^{3+}, Pd^{2+} Zn^{2+}, Au^{+}, and Au^{3+} also did not give any molecular ions corresponding to the complexes. Cr^{2+}, Fe^{2+}, Fe^{3+}, and Cd^{2+} showed complex ion peaks with moderate intensities. Only five transition-metal ions, Co^{2+}, Ni^{2+}, Cu^{2+}, Cu^{+}, and Ag^{+} exhibited very strong molecular ion peaks corresponding to the complexes with the cyclic thiosugars.

3.4 Study of Silver Complexation with Cyclic β-1,6-*S*-Linked Thiooligosaccharides by ^{1}H NMR Titration Experiments

3.4.1 Background

Silver isotope, ^{111}Ag, has found important applications in radio-immumnotherapy due to its very favorable decay properties such as medium half-life, convenient β-energy and low percentage of accompanying γ-emission [180-182]. However, silver (I) is known to be a very labile metal center and undergo fast trans-metalation processes to other competing coordination sites frequently existing in biological system. It has been found that thiacrown ethers can encapsulate silver (I) center and prevent the fast ligand exchange process, therefore allowing the silver center to be stable under *in vivo* conditions for days.

The strong binding property of cyclic β-1,6-*S*-linked thiooligosaccharides with silver ions demonstrated by ESI-MS screening experiment had been very encouraging. We decided to further investigate the Ag (I) complexation behavior with these novel carbohydrate-based macrocycles. Specifically, ^{1}H NMR titration technique was used to probe the binding sites and the binding stoichiometries.

3.4.2 ^{1}H NMR Titration Experiments

Cyclic thiooligosaccharide solutions (host molecule solutions) were prepared individually by dissolving about 1.0 mg of each sugar in 1.0 mL of D_2O in a vial. Then 0.1 mL of sugar solution was diluted into 0.7 mL of D_2O in an NMR tube. The concentration of the cyclic sugar in the NMR tube was 0.220 mM for cyclic trisaccharide **2**, 0.187 mM for tetrasaccharide **3** and 0.120 mM for pentasaccharide **4**. Ag (I) solution was prepared by dissolving 1.2 mg of dry $AgClO_4$ powder in 10 mL of D_2O and used as the guest solution for NMR titration. 10 μL of Ag (I) solution was added into each cyclic thiooligosaccharide solution in the NMR tube and mixed by ultrasonication. Each mixture was subjected to ^{1}H NMR measurement. Addition of Ag (I) solution was continued at 10 μL intervals until the chemical shifts of ring protons reached maximum and remained stable.

Silver ion complexation with all three cyclic thiosugars was found to be equilibrated within a few minutes. This was demonstrated by the stability of NMR spectra, which remained constant over an extended period of time.

3.4.3 Silver (I) Complexation with Cyclic Trisaccharide

The Ag (I)-induced ^{1}H NMR spectra of cyclic trisaccharide **2** exhibited one single set of proton signals for the glucopyranoside unit, demonstrating that the complexed molecule still maintained symmetry (**Figure 3.3**). Upon complexation with silver ions, H-6a and H-6b shifted to lower field while H-5 and H-2 showed limited downfield shifts. The NMR signals of H-5, H-2, H-6a and H-6b became broadened on addition of Ag (I)

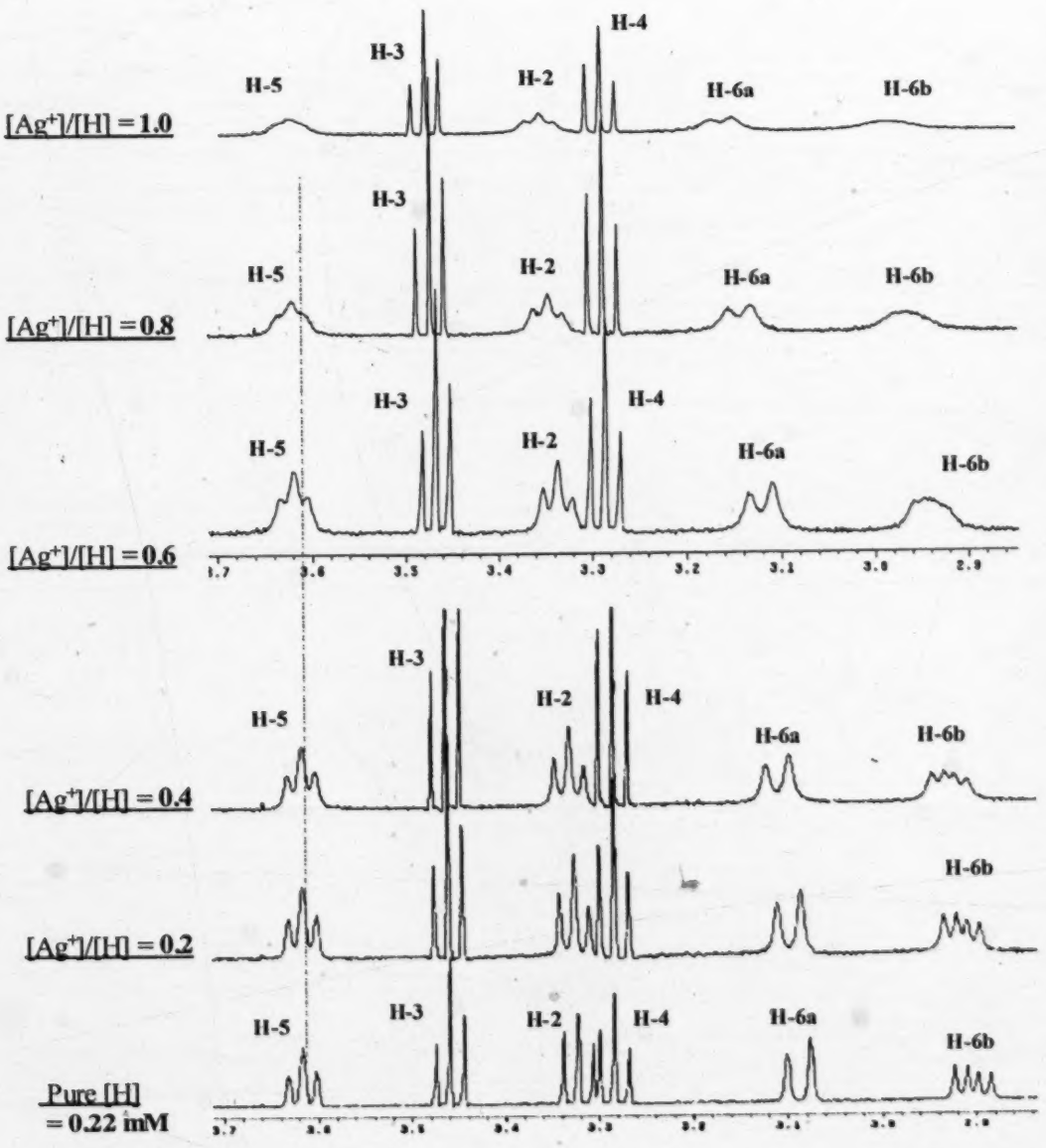


Figure 3.2 Induced ^1H NMR shifts of cyclic trisaccharide **2** by adding Ag (I) D_2O solution at 27 °C. [H] = total cyclic sugar concentration. (free cyclic sugar plus complexed cyclic sugar at equilibrium)

solution, while the chemical shifts and peak shapes of H-3 and H-4 remained unchanged. No significant change in coupling constants was observed for all these proton resonances.

The magnitude of these downfield shifts suggested that the complexation occurred near protons H-6a, H-6b, H-5 and H-2 in cyclic trisaccharide **2**. This implied that the silver ion was positioned in the center of the macrocyclic component of cyclic trisaccharide, since H-3 and H-4 sit at the peripheral rim of the macrocycle and were unaffected by the positive-charged silver ions (**Figure 3.3**).

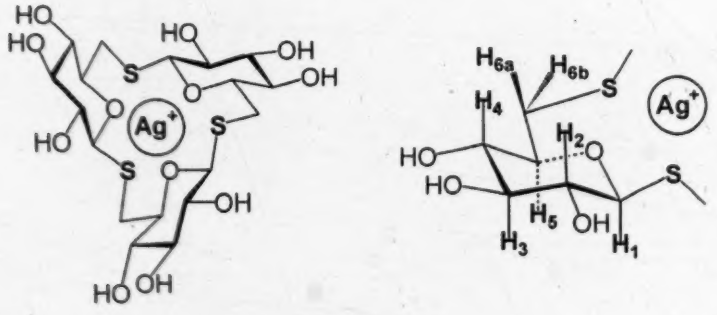


Figure 3.3 Schematic representation of Ag (I) complexes with cyclic sugars.

One possible explanation for the signal broadening of H-5, H-2, H-6a and H-6b is that the rapid interconversion of different conformations of free trisaccharide **2** was hindered by the presence of Ag (I) ions, which was interacting with sulfur atoms in the ring system. The conformational isomers of this Ag (I)-bounded macrocycle were slowly interexchanging with each other at room temperature. The coalescence of the proton

signals from these conformational isomers resulted in the broad peaks in the NMR spectra.

A typical titration curve showing the relationship between the induced chemical shift change and amount of guest Ag (I) added relative to total host molecule ($\Delta\delta$ vs. $[Ag^+]_{total}/[H]_{total}$) is depicted in **Figure 3.4** for cyclic trisaccharide **2**. The chemical shift

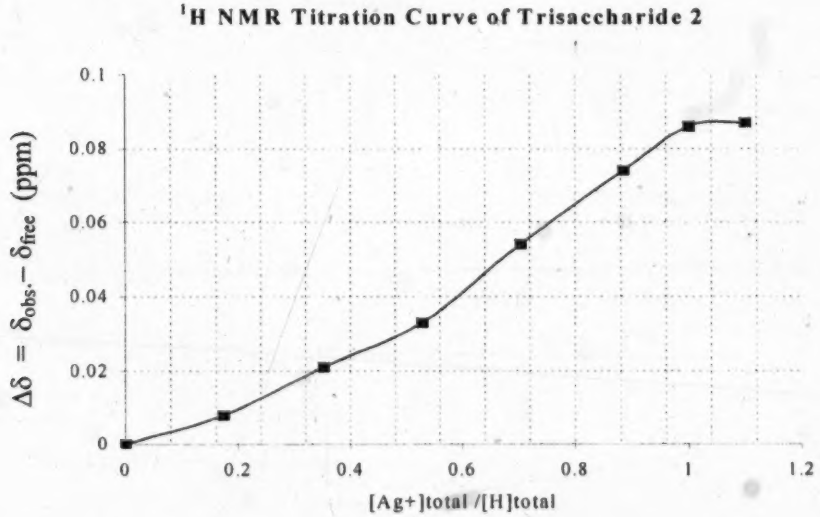


Figure 3.4 ^{1}H NMR titration curve of cyclic trisaccharide **2**.
(The chemical shift changes of H-6a were recorded in the titration.)

change of H-6a was recorded in the titration process. The $\Delta\delta$ (δobs.– δfree) gradually increased as Ag (I) was added; and the titration curve leveled out upon the addition of 1 eq. of Ag (I) per host molecule. This indicated a 1:1 stoichiometry of silver complexation with cyclic trisaccharide **2**.

3.4.4 Silver (I) Complexation with Cyclic Tetrasaccharide 3

Similar to the NMR titration experiment for trisaccharide **2**, the induced NMR spectra shifts also exhibited symmetrical structure for the complexed tetrasaccharide **3**. H-3 and H-4 slightly shifted downfield upon the addition of Ag (I) solution **(Figure 3.5).** The coupling pattern and coupling constants of the glucopyranoside protons remained unchanged upon the complexation.

No proton signal broadening was observed in the induced spectra of cyclic tetrasaccharide, indicating that the tetrasaccharide complex is more flexible than cyclic trisaccharide complex. Chemical shift changes of H-5 (0.10 ppm), H-2 (0.12 ppm) and H-6a (0.17 ppm) were more significant in the case of tetrasaccharide, compared to those in cyclic trisaccharide. H-5 showed relatively large upfield shift while H-2 and H-6a shifted downfield. The upfield shifting of H-5 and H-6b in the Ag (I) complex was probably because of these protons' being shielded by the neighboring sulfur atoms upon complexation.

The NMR titration curve of tetrasaccharide **3** is shown in **Figure 3.6**. The titration curve went up more rapidly than that of trisaccharide **2**, and leveled out when the ratio of total concentrations of cyclic sugar and metal ions reached the ratio of 1:1, again indicating a 1:1 binding stoichiometry.

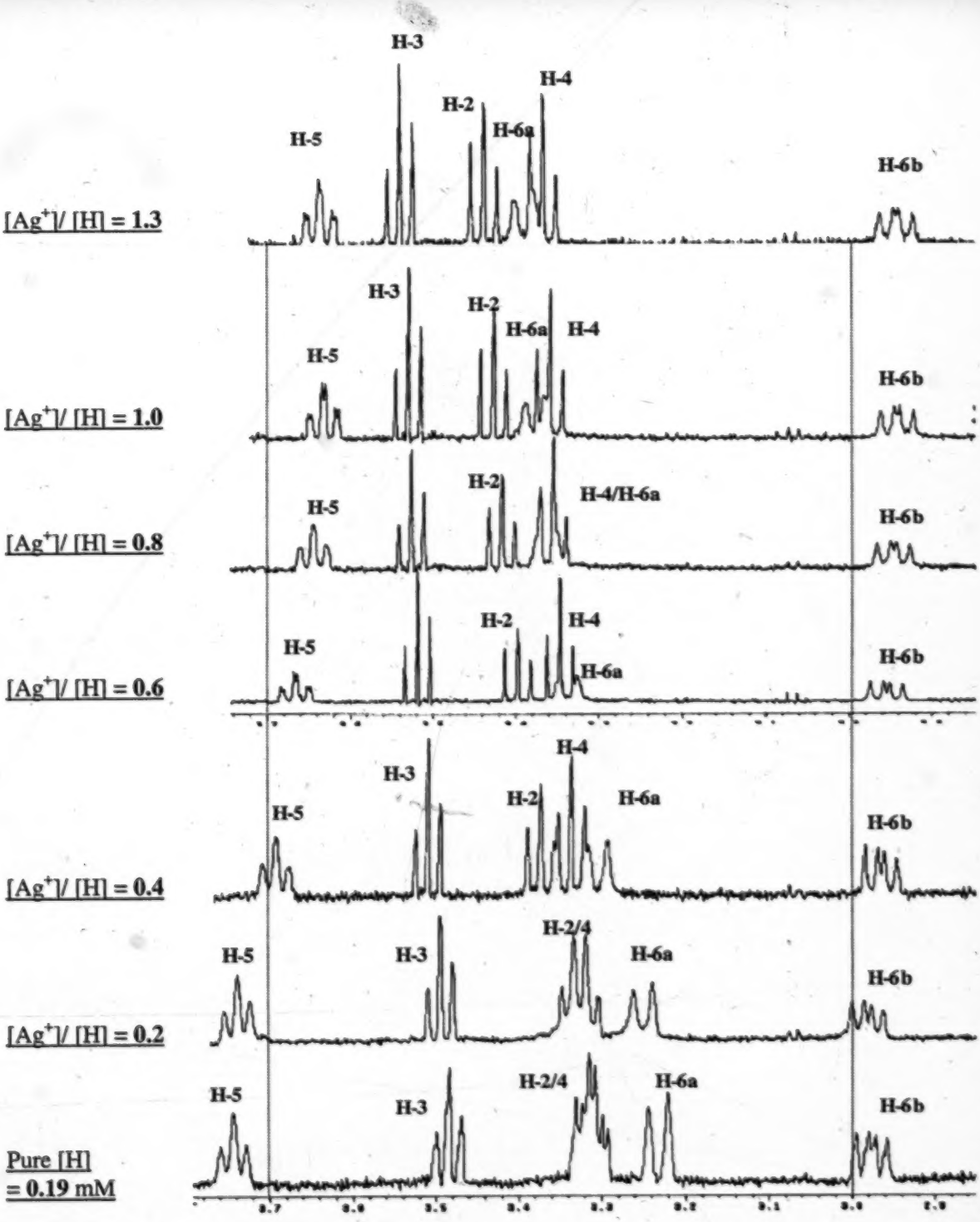


Figure 3.5 Induced ^{1}H NMR shifts of cyclic tetrasaccharide **3**, by adding Ag (I) D_2O solution at 27 °C. [H] = total concentration of cyclic sugar (free cyclic sugar plus complexed cyclic sugar at equilibrium)

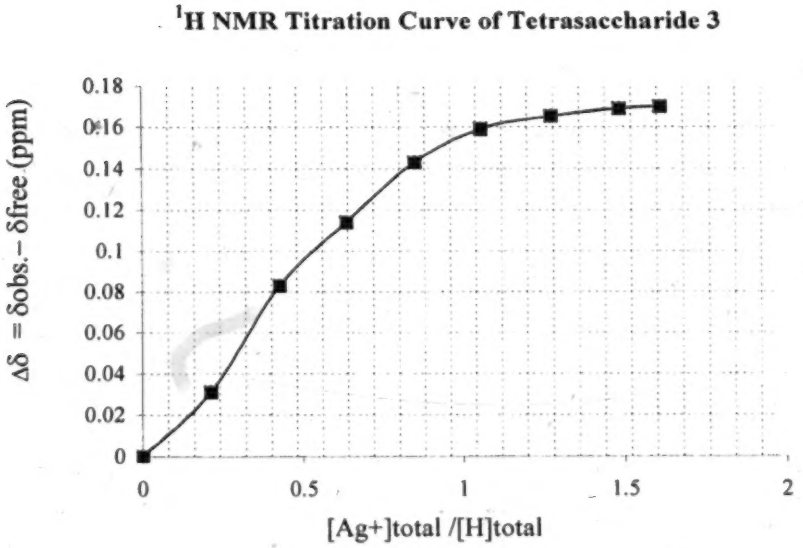


Figure 3.6 ^{1}H NMR titration curve of cyclic tetrasaccharide **3**.
(The induced chemical shift changes of proton H-6a were recorded.)

3.4.5 Silver (I) Complexation with Cyclic Pentasaccharide 4

The ^{1}H NMR spectrum of the cyclic pentasaccharide exhibited one single proton set of glucopyranose units as the same as was the case for the tri- and tetrasaccharides (**Figure 3.7**). Despite this similarity, the induced NMR spectrum for pentasaccharide **4** displayed a quite different and complicated pattern, possibly due to the structural flexibility of this large-sized macrocyclic ring. Chemical shift changes were observed for all the protons on the glucopyranose units, including H-3 and H-4, which remained unchanged in the cases of cyclic tri- and tetrasaccharide. H-6a and H-6b shifted downfield 0.18 and 0.20 ppm, respectively upon the addition of Ag (I) solution.

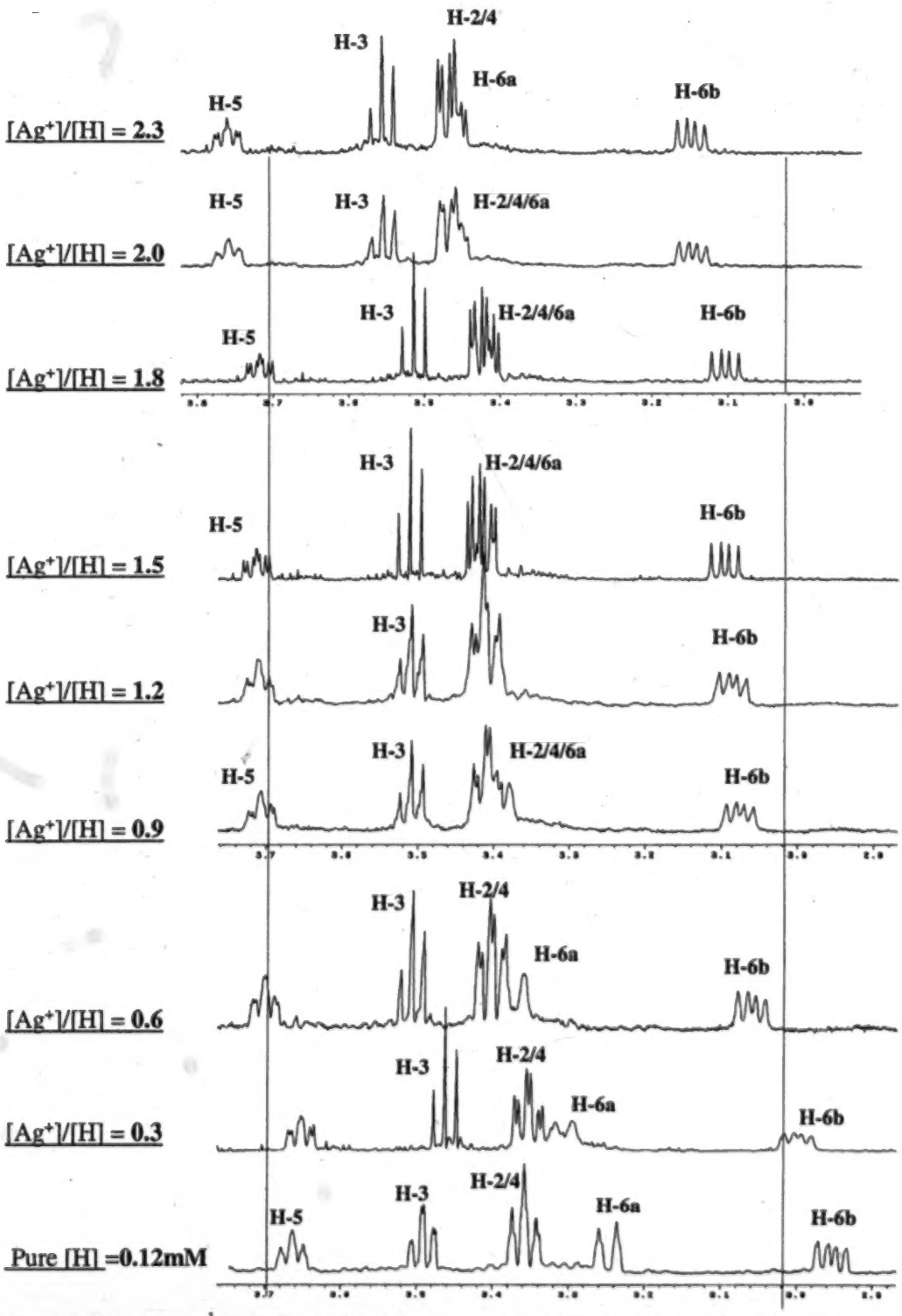


Figure 3.7 Induced ^{1}H NMR shifts of cyclic pentasaccharide **4**, by adding Ag (I) D_2O solution at 27 °C. [H] = total cyclic sugar concentration (free cyclic sugar plus complexed cyclic sugars at equilibrium)

In the process of the Ag (I) titration, H-5, H-3, H-2 and H-4 first shifted upfield when the ratio $[Ag^+]_{total}$ / $[H]_{total}$ was about 0.3. As more Ag (I) solution was added, these peaks shifted toward downfield gradually. The phenomenon of up and downfield shifting of these proton signals during the titration is probably due to a combination of de-shielding effect from positively charged silver ion and shielding effect from the neighboring sulfur atoms. There was no further change in chemical shift once the ratio reached 2:1 and this may suggest the formation of a complex with two Ag (I) ions and one cyclic pentasaccharide. The NMR titration curve of pentasaccharide **4** is shown in **Figure 3.8**.

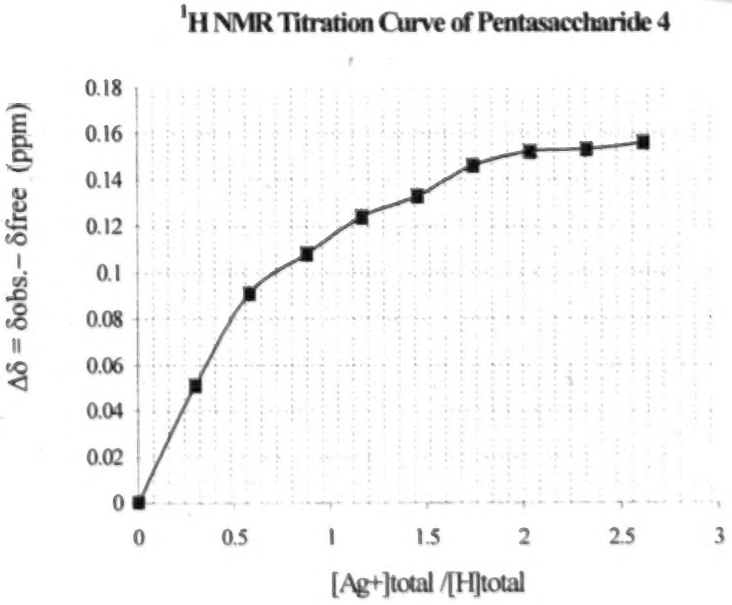


Figure 3.8 ^{1}H NMR titration curve of cyclic pentasaccharide **4**.
(The induced chemical shift changes of proton H-6b were recorded in the titration.)

3.5 Conclusion

The binding capabilities of three cyclic thio sugars with over 25 metal ions were screened by ESI-MS technique. Five transition-metal ions, Co (II), Ni (II), Cu (II), Cd (II), and Ag (I), were found to strongly bind with the cyclic β-1,6-*S*-glucopyranosides. Heavy metal ions, Pb (II) and Hg (II), which are commonly expected to bind strongly to sulfur atoms, showed no binding. NMR titration experiment revealed the formation of Ag (I) complexes with the novel carbohydrate-based macrocycles, providing valuable information on the binding stoichiometry and possible binding sites.

Chapter 4

Attempted Synthesis of β-1, 4-*S*-Linked Cycloglucopyranosides

4.1 Introduction

The ability of cyclodextrins to form unique inclusion complexes with a large variety of substances is derived from their unique, bucket-like structures. These rigid macrocycles are composed of homogenous glucopyranosides joined to each other by glycosidic linkages involving axial C-1-O and equatorial C-4-O (i.e. α-1,4-O glucopyranosyl linkage). All the glucose units of cyclodextrins adopt substantially undistorted $^4C_1$ chair conformations. The total synthesis of 1,4-linked cyclic oligosaccharides is of particular interest, because it is directed toward the formation of large ring molecules which are expected to have well-defined internal cavities and consequently, to exhibit binding of appropriate substrates. This chapter describes our attempted synthesis of β-1,4-*S*-linked cycloglucopyranosides using the methodology developed in the synthesis of cyclic β-1,6-*S*-glucopyranosides.

In contrast to cyclic sugars containing flexible 1,6-glycosidic linkages that have enough conformational freedom for the incorporation of all kinds of monosaccharide residues, the 1,4-linked cyclic oligosaccharides have certain structural restraints for the monosaccharide residue. It is generally accepted that 1,4-linked cyclic sugars with stable pyranoside chair conformations ($^1C_4$ or $^4C_1$) requires a combination of axial/equatorial orientations at C-1/C-4 (**Figure 4.1**). In other words, the monosaccharide residues have

to carry one axial and one equatorial bond respectively, at either C-1 and C-4, or C-4 and C-1, to be capable of formation of a cycle.

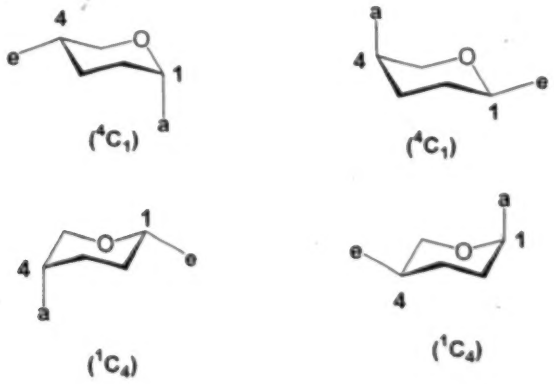


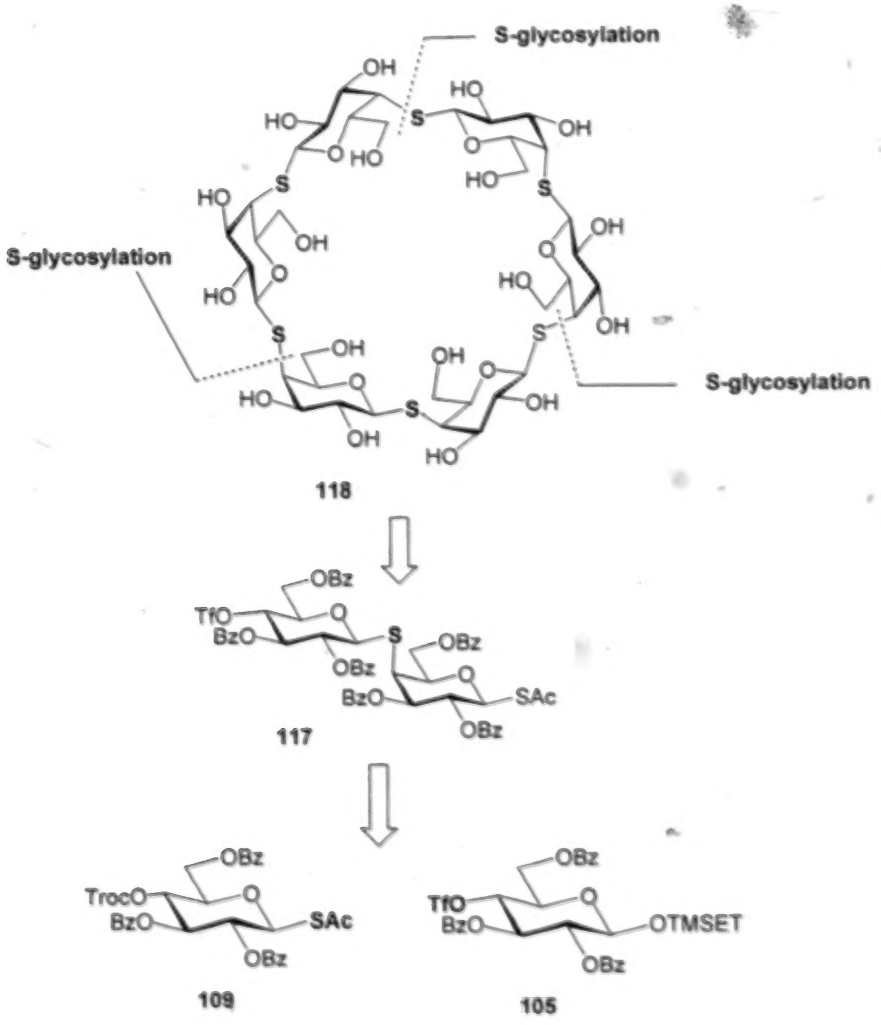
Figure 4.1 Structural considerations for the repeating units of 1,4 –linked cyclic sugars. (Four possible relative configurations of pyranoside ring with stable $^4C_1$ and $^1C_4$ conformations)

Based on the materials we had in hand, we decided to set β-1,4-*S*-linked cycloglucopyranosides **118** as our next synthetic target (**Scheme 4.1**). The synthesis of this novel type cyclic sugar would provide us with the opportunities to explore its substantially different properties derived from its unique cavity, which containing six larger nucleophilic and hydrophobic sulfur atoms, compared to the native O-linked cyclic sugars.

4.2 Retrosynthetic Analysis

A polycondensation approach was attempted in order to assemble the target molecule **118** rapidly from a suitable monomer, although polymerization of the monomer would likely to be a competing process **(Scheme 4.1)**.

The choice of the polycondensation approach was based on two observations: (1) similar cyclization approaches have been successfully applied for the synthesis of unnatural cyclic *O*-linked oligosaccharides via *O*-glycosidations; (2) polycondensation provides the chance to synthesize a series of cyclic sugars in one pot. In case our desired molecule, cyclic hexaglucopyranoside **118** with 1,4-S-linkages, is sterically too rigid to form, the polycondensation approach would still lead to the higher cyclic macrocycles containing more than six homogenous residues.



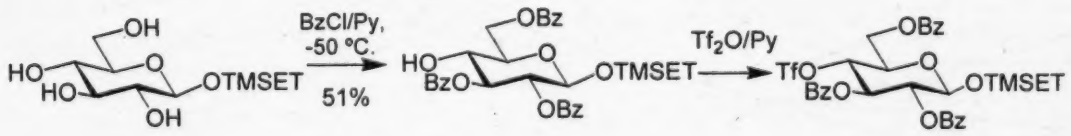
Scheme 4.1 Retrosynthetic analysis of target molecule **118.**

The conventional and effective method to establish linear thiooligosaccharides containing 1,4-*S*-linkages is the S_N2-displacement of triflates by anomeric thiolates. Thus, disaccharide monomer **117** was designed, bearing an anomeric thioacetate at the reducing end and a triflate on 4-position at the non-reducing end. It was hoped that intermediate **117** would undergo a trimerization process to give the desired free macrocycle **118** after the removal of the benzoyl groups.

Monosaccharide **109** and **105** were regarded as versatile building blocks for the synthesis of disaccharide monomer **117**. Both **109** and **105** could be conveniently prepared from a TMSET glucopyranoside **(Scheme 4.1)**.

4.3 Preparation of Thio Glucosyl Donor

Our synthesis of building block **105**, 2-(trimethylsilyl)ethyl 2, 3, 6-tri-*O*-benzoyl-4-*O*-trifluoromethanesulfonyl-β-D-glucopyranoside, started from the treatment of the TMSET glucoside **6** with 3 eq. of BzCl in pyridine at –50 °C (**Scheme 4.2**).



6 **104** **105**

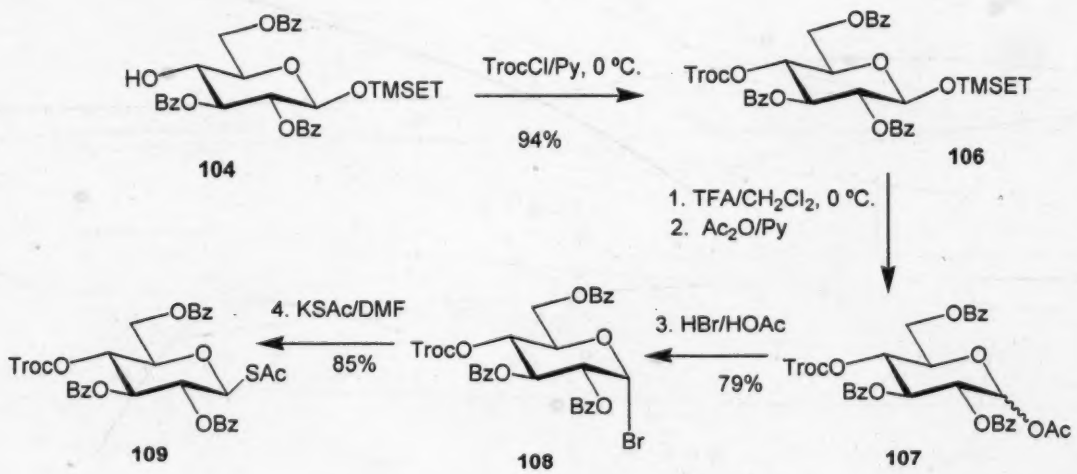
Scheme 4.2 Synthesis of thio glucosyl donor **105.**

The OH-4 of the TMSET glycoside **6** has the lowest reactivity toward the benzoylation among the hydroxyl groups. A mixture was obtained with the desired

compound **104** as the major product. The reaction was quenched with methanol when the formation of **104** reached its optimum and gave a 51% yield after chromatographic purification. Esterification of 4-OH group of **104** with trifluoromethanesulfonic anhydride afforded triflate **105** in a quantitative yield.

4.4 Preparation of Thio Glucosyl Acceptor

The initial step in the synthesis of glycosyl acceptor **109** was to protect the OH-4 of **104** with a Troc group (**Scheme 4.3**). With the Troc group in position, the TMSET glucopyranoside **106** was successfully converted into the glucopyranosyl thioacetate **109**, following the same four-step procedure described in the chapter 2. TMSET glucopyranoside **106** was first treated with 50% TFA to give a hemiacetal, followed by acetylation of the anomeric OH group with acetic anhydride in pyridine. Bromination of

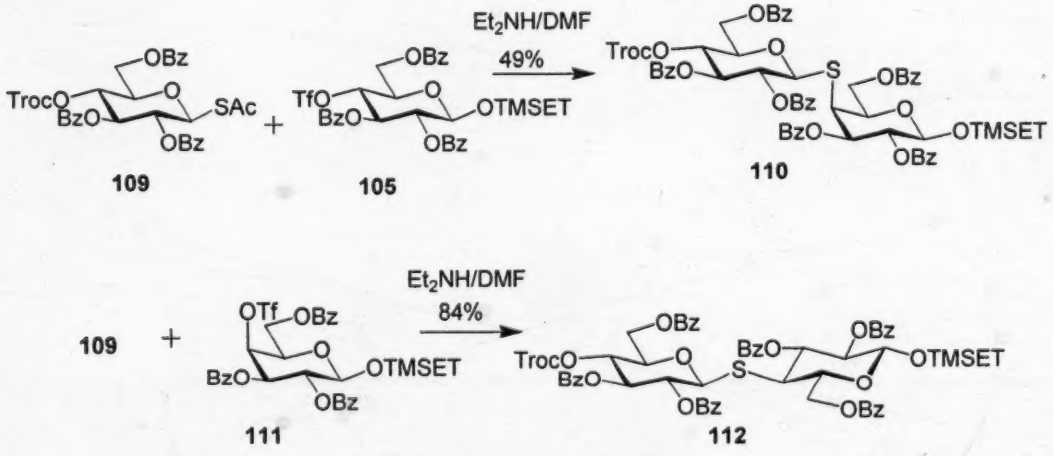


Scheme 4.3 Synthesis of thio glucosyl acceptor **109.**

107 with hydrogen bromide in acetic acid provided bromo-sugar **108**. Without further purification, **108** was then treated with KSAc in DMF to give the desired 1-thioacetate **109** in 85% yield.

4.5 Synthesis of Disaccharide Monomer 115

The first glycosyl coupling reaction to construct a 1,4-*S*-linkage was attempted between thioacetate **109** and triflate **105** (**Scheme 4.4**). This *in situ* coupling reaction was carried out in DMF in the presence of Et_2NH. It produced the expected β-1,4-*S*-linked disaccharide **110** in only a moderate yield of 49%. We realized that high efficiency of the thioglycosyl coupling reaction is crucial for a reasonable yield of macrocyclization process, which involves several simultaneous intermolecular thioglycosyl coupling reactions. Thus other conditions (different solvents, temperatures, durations, etc.) were examined in an attempt to improve the yield, but all were unsuccessful.

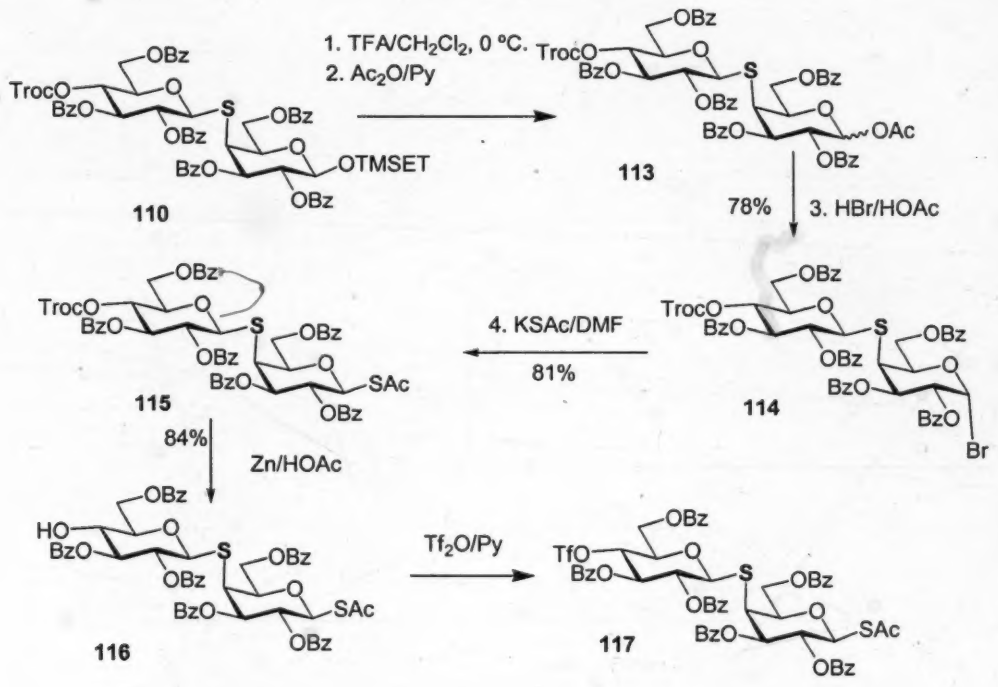


Scheme 4.4 1,4-Thioglycosylation.

We suspected that the backside of 4-triflate of **105** was sterically hindered by the two benzoates at both 3- and 6-positions. In our previous study, a similar coupling reaction was carried out between **109** and 2,3,6-tri-*O*-benzoyl-4-*O*-trifluoromethanesulfonyl-galactopyranoside **111**, and it gave the disaccharide **112** in 84% yield. This implied that the low efficiency of coupling between **109** and **105** was due to the inherent structure of building block **105.** In galactopyranoside **111**, the 4-triflate and the benzoates at 3- and 6-positions are on the same side of the pyranoside ring, thus the backside of the triflate is more accessible for the nucleophilic displacement. It was tempting to suggest that preparation of cyclic sugars with β-1,4-*S*-galato linkages would be more difficult than that of cyclic α-1,4-*S*-gluco linkages, which are constructed using 4-*O*-trifluoromethanesulfonyl galactopyranosides as building blocks.

We noticed that the Troc group in **109** remained intact under the mild basic conditions, in contrast to our initial anticipation (**Scheme 4.4**). The stability of 4-Troc group under this condition simplified the purification process, and also avoided one synthetic step to reinstall the Troc group if it fell off.

Our next step was to convert the anomeric TMSET group in disaccharide **110** into the anomeric thioacetate in disaccharide **115** (**Scheme 4.5**), again following the optimized four-step procedure described in Section 2.8 of the chapter 2. The transformation was completed smoothly in overall 59% yield from **110**. Next, the Troc group in **115** was removed as usual by zinc in acetic acid in 86% yield. The triflation of OH-4 in the disaccharide **116** gave the desired disaccharide monomer **117** quantitatively.

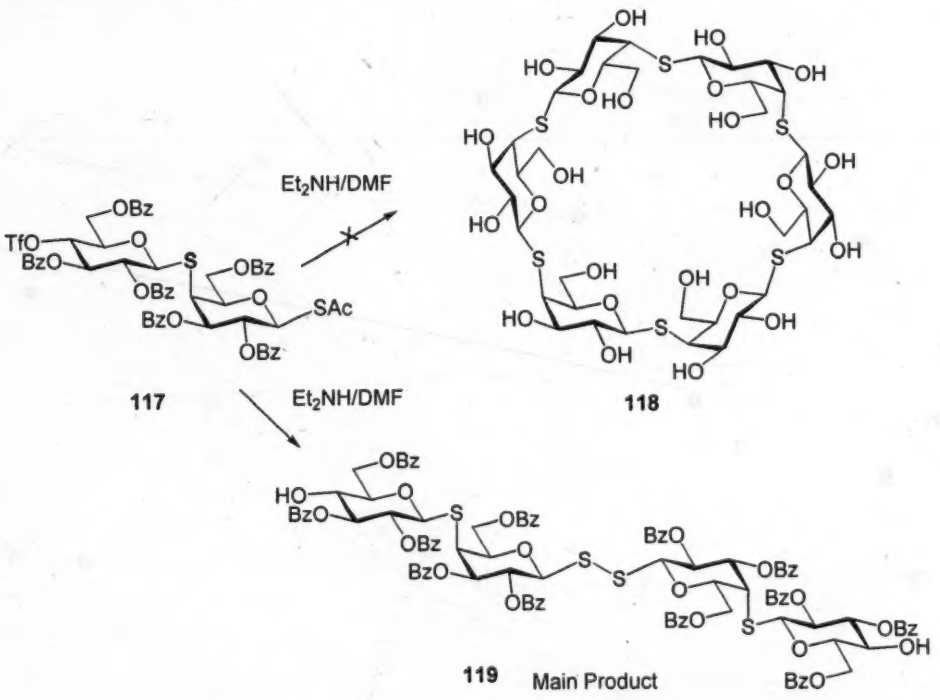


Scheme 4.5 Synthesis of disaccharide monomer **117**.

4.6 Attempted Cyclo-oligomerization of 117

The bifunctional disaccharide **117** was expected to serve as a monomer in the crucial trimerization process for the synthesis of cyclic sugar (**Scheme 4.6**). The reaction was performed at a high concentration (0.1 M) with the intention to promote the intermolecular reaction. To a solution of compound **117** (112 mg, 0.1 mmol) in 1.0 mL of DMF was added Et_2NH (75 μL, 0.7 mmol) at 0 °C. The solution was allowed to reach room temperature and stirred overnight. The reaction mixture was washed, concentrated under vacuum and then subjected to the ESI-MS. Unfortunately no trace of desired cyclic sugar **118** was detected by ESI-MS. Instead, the linear tetrasaccharide disulfide

119 was isolated as the main product in the complex mixture. Apparently, disulfide **119** resulted from the oxidation of anomeric thiolates and the decomposition of triflate groups in precursor **117**. It was tempting to suggest that the relatively low efficiency of intermolecular thioglycosylation reaction between 4-triflate and 1-thioacetate of disaccharide monomer **117** was probably the key factor for the unsuccessful macrocyclization.



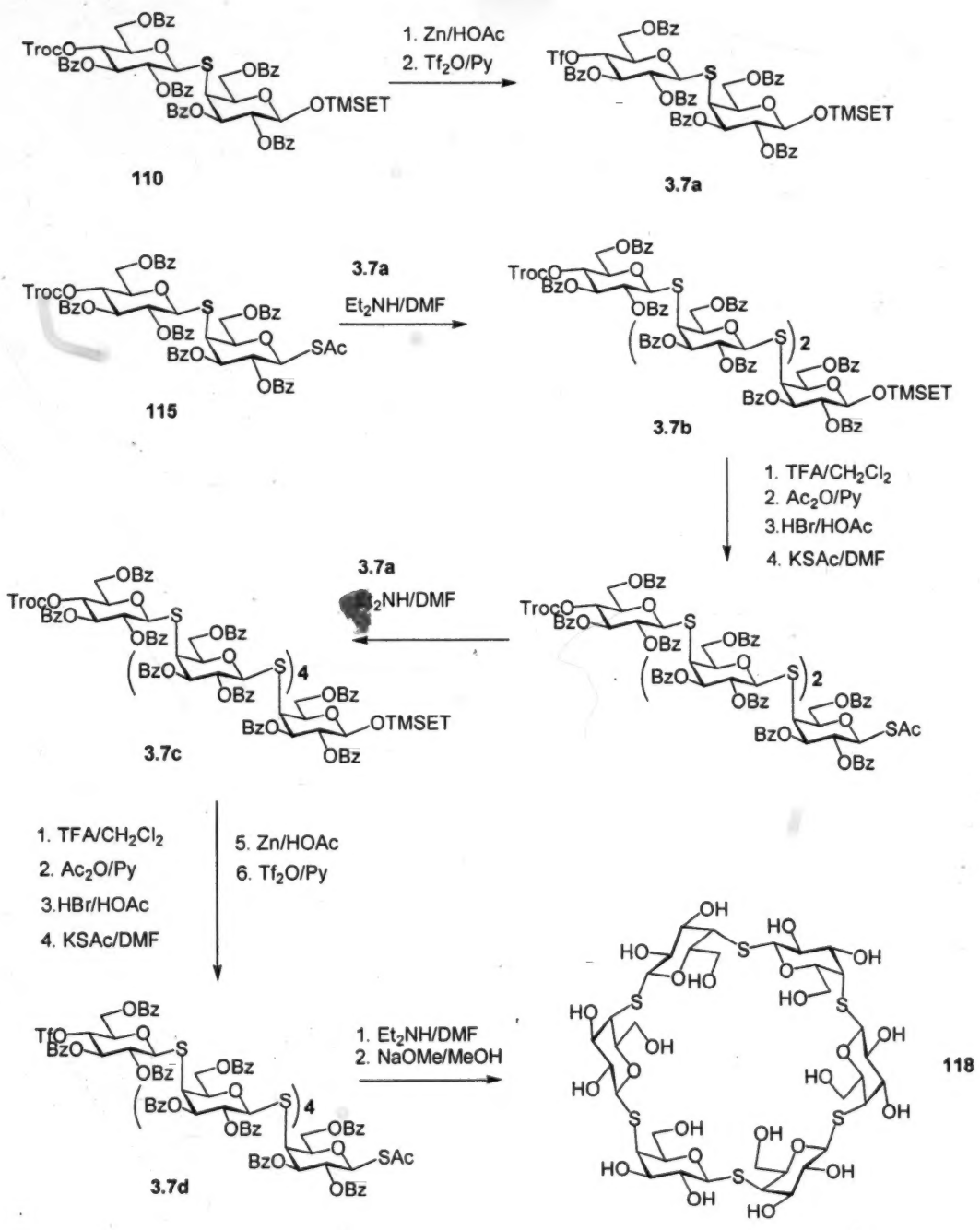
Scheme 4.6 Polycondensation of disaccharide monomer **117**.

In summary, our rapid preparation of disaccharide monomer **117** indicates the feasibility of our synthetic approach to prepare bifunctionalized precursors, which could be potentially used to synthesize the cyclic thiooligosaccharides with other type of thio-linkages.

4.7 Future Perspectives

The shortcoming of polycondensation approach to synthesize cyclic sugars is that it often leads to polymerization products instead of the desired cyclic compounds. There are no reliable and universal methods specifically to produce cyclic products, although in literature very few examples utilizing template-directed or structure-directed protocol to promote the cyclization process have been explored. However, for the polycondensation of a thio-linked disaccharide monomer, suitable transition-metal ions could be potentially used to selectively bind glycosidic sulfur atoms and have the monomers pre-organized. This might help to facilitate the macrocyclization process.

Although the polycondensation approach for the synthesis of 1,4-S-linked cyclic sugar was unsuccessful, the alternative approach, which involves an intramolecular thioglycosylation of bifunctional linear 1,4-*S*-hexaglucopyranoside **3.7d** under high-dilution condition, could be explored. Followed the same methodology developed in Chapter 2, a disaccharide building block **3.7a** first could be easily prepared from the disaccharide **110** in two steps (**Scheme 3.7**). The desired linear precursor **3.7d** would be derived from two iterative coupling between disaccharide **115** and **3.7a**, followed by bifuntionalization of the resulting hexasaccharide **3.7**. All of these modifications could follow the same procedures developed for the synthesis of cyclic 1,6-*S*-linked oligosaccharides.



Scheme 4.7 Proposed scheme for the synthesis of target cyclic sugar **118** *via* bifunctionalized linear thiooligosaccharide **3.7d.**

Chapter 5

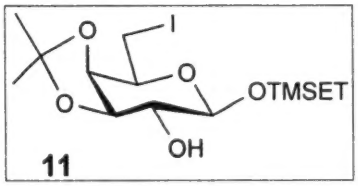
Experimental

General Methods

Analytical thin layer chromatography (TLC) was performed on silica gel 60-F254 (Merck) with detection by quenching of fluorescence or by charring with 5% H_2SO_4 in ethanol. Column chromatography was performed on Silica Gel 60 (E. Merck, 40-63 μm) and solvents were distilled. All commercial reagents were used a supplied unless otherwise stated. Milliex-GV (0.22 μm) filter units were from Millipore. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 22 °C. ^{1}H NMR spectra were recorded at either 400, 500 or 600 MHz (Varian Unity), and are referenced to internal standards of the residual protonated solvent peaks; δ_H 4.78 ppm for solutions in CD_3OD or 0.1% external acetone (δ_H 2.225 ppm) for solutions in D_2O. ^{13}C NMR spectra were recorded at 100 or 150 MHz and are referenced to internal $CDCl_3$ (δ_C 77.0 ppm) or D_2O to external acetone (δ_C 31.07 ppm). All coupling constants are reported as observed splitting of signals using first order analysis. Mass spectrometric analysis was performed by positive mode electrospray ionization on a Micromass ZabSpec Hybrid Sector-TOF.

2-(Trimethylsilyl)ethyl 6-O-deoxy-6-iodo-3,4-O-isopropylidene-β-D-galactopyranoside

(11)

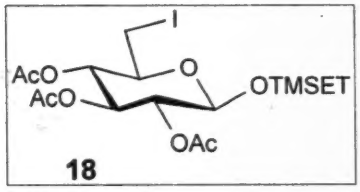


11

To a solution of **10** (2.50 g, 7.8 mmol) in toluene (100 mL) was added triphenylphosphine (3.88 g, 14.8 mmol), imidazole (2.65 g, 39 mmol) and iodine (6.9 g, 27.3 mmol). The mixture was stirred for 20 min at 60 °C under argon. The mixture was filtered through a celite pad, concentrated and then subjected to column chromatography (hexane/ethyl acetate: 3:1), which gave compound **11** (2.89 g, 86%) as a white solid.

$[\alpha]_D$ 22.7° (c 1.2, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 4.31 (dd, 1H, J = 5.5, 2.0 Hz, H-4), 4.17 (d, 1H, J = 8.0 Hz, H-1), 4.07 (dd, 1H, J = 7.0, 5.5 Hz, H-3), 4.03 (dt, 1H, J = 10.0, 7.0 Hz, $O\underline{CH_2}CH_2SiMe_3$), 3.90 (dt, 1H, J = 7.0, 2.0 Hz, H-5), 3.59 (dt, 1H, J = 10.0, 7.0 Hz, $O\underline{CH_2}CH_2SiMe_3$), 3.51 (t, 1H, J = 8.0 Hz, H-2), 3.40 (m, 2H, H-6a, H-6b), 1.51, 1.35 (2s, 6H, Me_2C), 0.90-0.80 (m, 2H, $OCH_2C\underline{H_2}SiMe_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 110.20 ($\underline{C}Me_2$), 101.65, 78.72, 73.86, 73.78, 73.45, 67.38 ($O\underline{C}H_2CH_2SiMe_3$), 28.07, 26.25 ($\underline{Me_2}C$), 18.21 ($OCH_2\underline{C}H_2SiMe_3$), 1.87 (C-6), -1.42 ($OCH_2CH_2Si\underline{Me_3}$). HRMS (ESI): Calc. for $C_{14}H_{27}IO_5SiNa$ $[M+Na^+]$ 453.0570, observed 453.0565.

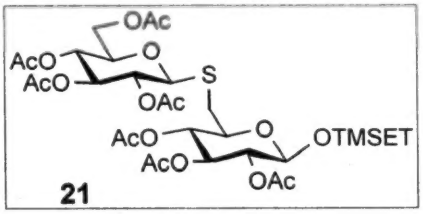
2-(Trimethylsilyl)ethyl 2,3,4-tri-O-acetyl-6-deoxy-6-iodo-β-D-glucopyranoside (18)



18

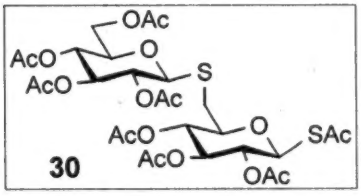
A mixture of 2-(trimethylsily)ethyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside **17** (7.18 g, 17.7 mmol), triphenylphosphine (8.82 g, 33.6 mmol), imidazole (6.02 g, 88.5 mmol), and iodine in toluene (150 mL) was vigorously stirred at 60 °C for 30 min. The mixture was filtered through a celite pad, concentrated and then subjected to column chromatography (hexane/ethyl acetate: 5:1→ 3:1), which gave title compound **18** (8.67 g, 95%) as a solid. $[\alpha]_D$ - 3.9° (c 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.16 (t, 1H, J = 9.5 Hz, H-3), 4.94 (dd, 1H, J = 8.0, 9.5 Hz, H-2), 4.85 (t, 1H, J = 9.5 Hz, H-4), 4.51 (d, 1H, J = 8.0 Hz, H-1), 4.01 (dt, 1H, $OCH_2CH_2SiMe_3$), 3.63 (dt, 1H, J = 10.0, 7.0 Hz, $OCH_2CH_2SiMe_3$), 3.50 (dt, 1H, J = 9.0, 3.0 Hz, H-5), 3.26 (dd, 1H, J = 11.0, 3.0 Hz, H-6a), 3.12 (dd, 1H, J = 11.0, 9.0 Hz, H-6b), 2.03, 2.00, 1.97 (3s, 9H, $COCH_3$), 0.90 (m, 2H, $OCH_2CH_2SiMe_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.18, 169.47, 169.21, 99.85, 73.52, 72.59, 72.27, 71.66, 67.53, 20.66, 20.55, 17.83, 2.99, -1.43. HRMS (ESI): Calc. for $C_{17}H_{29}IO_8SiNa$ $[M+Na^+]$ 539. 0574, observed 539. 0547.

2-(Trimethylsilyl)ethyl S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranoside (21)



A mixture of glucosyl thioacetate **22** (15.60 g, 15.55 mmol) and iodide **18** (6.98 g, 13.52 mmol) in dry DMF (100 mL) was stirred at –20 °C for 30 min. Diethylamine (10 mL, 95 mmol) was then added under Ar atmosphere. The reaction mixture was stirred for a further 1 h below 0 °C then allowed warming to rt. Diethylamine was removed under vacuum. The residue was diluted with CH_2Cl_2, washed with dilute HCl (1N, 50 mL), water (3 × 100 mL), dried over Na_2SO_4, and concentrated. Column chromatography (hexane/ethyl acetate: 3:1) gave disaccharide **21** (8.45 g, 83%). $[\alpha]_D$ - 13.2° (c 1.4, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.16 (t, 1H, J = 9.5 Hz, H-3), 5.14 (t, 1H, J = 9.5 Hz, H-3'), 5.03 (t, 1H, J = 10.0 Hz, H-4'), 4.95 (dd, 1H, J = 6.0, 10.0 Hz, H-2'), 4.92 (dd, 1H, J = 6.0, 9.0 Hz, H-2), 4.88 (t, 1H, J = 10.0 Hz, H-4), 4.61 (d, 1H, J = 10.0 Hz, H-1'), 4.48 (d, 1H, J = 8.0 Hz, H-1), 4.22 (dd, 1H, J = 5.0, 12.0 Hz, H-6a'), 4.12 (dd, 1H, J = 2.0, 12.0 Hz, H-6b'), 4.00 (dt, 1H, J = 6.0, 9.5 Hz, $OC\underline{H}_2CH_2SiMe_3$), 3.71-3.60 (m, 2H, H-5, H-5'), 3.56 (dt, 1H, J = 6.0, 9.5 Hz, $OC\underline{H}_2CH_2SiMe_3$), 2.81 (m, 2H, H-6a, H-6b) 2.04, 2.02, 2.00, 1.98, 1.97 (6s, 18H, $COCH_3$), 0.90 (m, 2H, $OCH_2C\underline{H}_2SiMe_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.45, 170.10, 169.872, 169.45, 169.25, 169.11, 169.10, 100.15, 83.69, 76.02, 74.12, 73.80, 72.90, 71.89, 71.53, 70.16, 68.30, 67.67, 62.12, 31.37, 20.84, 20.77, 20.78, 20.75, 20.68, 20.65, 20.64, 18.00, -1.23. HRMS (ESI): Calc. for $C_{31}H_{48}O_{17}SSiNa$ $[M+Na^+]$ 775.2279, observed 775.2278.

S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-1-S-acetyl-1,6-dithio-β-D-glucopyranose (30)



30

The TMSET disaccharide **21** (6.47 g, 8.59 mmol) was dissolved in dichloromethane (25 mL), CF_3COOH (25 mL) was added at 0 °C, and the mixture was stirred for 30 min. Toluene (100 mL) was added and then removed under vacuum. A second portion of toluene (60 mL) was added and removed, which gave the reducing disaccharide **28** sufficiently pure for the next step. HRMS (ESI): Calc. for **28**, $C_{26}H_{36}O_{17}SNa$ [M+Na$^+$]: 675.1571, observed 675.1570.

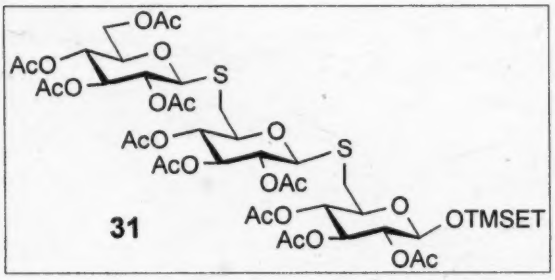
The hemiacetal **28** was then dissolved in pyridine (9 mL) and acetic anhydride (6 mL). After 3 h, the solution was poured into ice water and extracted with CH_2Cl_2 (100 mL) twice. The CH_2Cl_2 solution was washed with 1N HCl, H_2O, saturated $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated, affording compound **27**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.26 (d, 1H, J = 4.0 Hz, H-1, α-anomer), 5.69 (d, 1H, J = 10.0 Hz, H-1, β-anomer). α/β=2:1. HRMS (ESI): Calc. for **27**, $C_{28}H_{38}O_{18}SNa$ [M+Na$^+$]: 717.1677, observed 717.1673.

Crude disaccharide 1-acetate **27** was treated with 15 mL 33% HBr in acetic acid and stirred at 0 °C for 45 min and then poured into ice water and extracted with CH_2Cl_2 (2×50 mL), washed with H_2O, saturated $NaHCO_3$ solution, dried (Na_2SO_4), filtered and concentrated, affording disaccharide bromide **29** (4.82 g, 78%). $[\alpha]_D$ +62.5° (c 0.7,

$CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 6.57 (d, 1H, J = 4.0 Hz, H-1), 5.50 (t, 1H, J = 9.0 Hz, H-3), 5.19 (t, 1H, J = 9.0 Hz, H-3'), 5.07-5.02 (m, 2H, H-4, H-4'), 4.95 (t, 1H, J = 9.0 Hz, H-2'), 4.79 (dd, 1H, J = 4.0, 9.0 Hz, H-2), 4.55 (d, 1H, J = 7.0 Hz, H-1'), 4.29 (ddd, 1H, J = 4.0, 7.0, 12.0 Hz, H-5), 4.24 (dd, 1H, J = 5.0, 10.0 Hz, H-6a'), 4.12 (dd, 1H, J = 2.0, 12.0 Hz, H-6b'), 3.68 (ddd, 1H, J = 4.0, 7.0, 12.0 Hz, H-5'), 2.90-2.80 (m, 2H, H-6a, H-6b), 2.08, 2,07, 2.06, 2.05, 2.01, 2.00, 1.98 (7s, 3H each, $COCH_3$). HRMS (ESI): Calc. for $C_{26}H_{35}O_{16}SNaBr$ [$M+Na^+$] 737.0727, observed 737.0726.

Without further purification, bromide **29** (4.56 g, 6.37 mmol) was dissolved in dry DMF under Ar. Potassium thioacetate (2.55 g, 22.3 mmol) was added and the mixture was stirred at rt for 4 h. The residue was diluted with CH_2Cl_2 (100 mL), washed with dilute HCl (1N, 50 mL), water (2 × 50 mL), dried (Na_2SO_4), concentrated and purified by column chromatography (hexane/ethyl acetate: 3:2) on silica gel, which gave title product **30** (3.39 g, 75 %). $[\alpha]_D$ - 21.75° (c 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 5.24 (t, 1H, J = 9.0 Hz, H-3), 5.12 (t, 1H, J = 9.0 Hz, H-3'), 5.07 (dd, 1H, J = 9.0, 10.0 Hz, H-2), 5.02 (t, 1H, J = 10.0 Hz, H-4), 4.93-4.89 (2t, 2H, H-4, H-2), 4.22 (dd, 1H, J = 5.0, 12.0 Hz, H-6a'), 4.12 (dd, 1H, J = 2.0, 10.0 Hz, H-6b'), 3.82 (ddd, 1H, J = 3.0, 9.00, 10.0 Hz, H-5'), 3.60 (ddd, 1H, J = 2.0, 5.00, 7.0 Hz, H-5''), 2.80 (dd, 1H, J = 9.0, 15.0 Hz, H-6a), 2.64 (dd, 1H, J = 3.0, 15.0 Hz, H-6b), 2.41 (s, 3H, $SCOCH_3$), 2.08, 2.06, 2.03, 2.00, 1.97 (7s, 21H, $COCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.60, 170.52, 169.99, 169.82, 169.45, 169.40, 169.18, 82.89, 80.03, 79.97, 75.92, 73.86, 73.76, 71.33, 70.04, 69.00, 68.35, 62.07, 30.88, 30.23, 20.86, 20.73, 20.67, 20.66, 20.65, 20.64. Anal. Calcd.. for $C_{28}H_{38}O_{17}S_2$: C, 47.32; H, 5.39; S, 9.02; Found: C, 47.13; H, 5.18; S, 8.96.

2-(Trimethylsilyl)ethyl S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranoside (31)

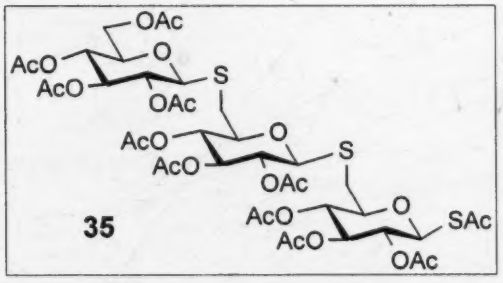


31

A mixture of disaccharide thioacetate **30** (3.07 g, 4.32 mmol) and 6-iodo monosaccharide **18** (2.00 g, 3.89 mmol) in dry DMF (25 mL) was stirred at –20 °C for 30 min. Diethylamine (3 mL) was then added under argon atmosphere. The reaction mixture was stirred for a further 1 h below 0 °C then allowed warming to rt. After 2 h, diethylamine was removed in vacuum. The residue was diluted with CH_2Cl_2, H_2O (2 × 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Column chromatography (hexane/ethyl acetate: 1:2) gave linear trisaccharide **31** (3.75 g, 81%). $[\alpha]_D$ + 4.8° (c 1.1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 5.19 (t, 1H, J = 9.0 Hz, H-3''), 5.18-5.13 (m, 2H, H-3', H-3), 5.06 (t, 1H, J = 9.5 Hz, H-4''), 4.97 (t, 1H, J = 9.0 Hz, H-2''), 4.94-4.86 (m, 4H, H-2, H-2', H-4', H-4), 4.63 (d, 1H, J = 10.0 Hz, H-1'), 4.59 (d, 1H, J = 10.0 Hz, H-1''), 4.52 (d, 1H, J = 8.0 Hz, H-1), 4.24 (dd, 1H, J = 5.0, 12.5 Hz, H-6a''), 4.15 (dd, 1H, J = 2.0, 12.5 Hz, H-6b''), 3.99 (dt, 1H, J = 6.0, 10.0 Hz, $OC\underline{H}_2CH_2SiMe_3$), 3.74-3.63 (m, 3H, H-5'', H-5', H-5), 3.57 (dt, 1H, J = 6.0, 10.0 Hz, $OC\underline{H}_2CH_2SiMe_3$), 2.87 (dd, 1H, J = 3.0, 14.0 Hz, H-6a'), 2.83-2.76 (m, 3H, H-6b', H-6a, H-6b), 0.90 (m, 2H, $OCH_2C\underline{H}_2SiMe_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.77, 168.42, 168.23, 168.20, 167.87, 167.79, 167.58, 167.51, 167.45, 167.43, 100.11, 83.70, 83.46, 77.70, 76.20, 74.12, 73.75, 73.64, 72.91, 71.74,

71.64, 71.60, 70.33, 69.76, 68.26, 67.65, 62.10, 31.76, 31.23, 20.88, 20.88, 20.80, 20.78, 20.76, 20.75, 20.69, 20.65, 17.95, -1.25. Anal. Calcd.. for $C_{43}H_{64}O_{24}S_2Si$: C, 48.85; H, 6.10; S, 6.07; Found: C, 48.63; H, 6.19; S, 6.25.

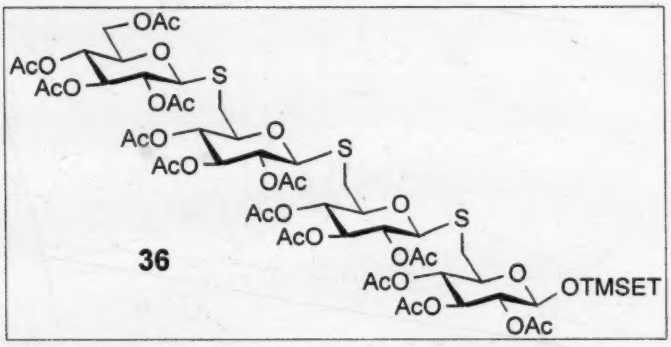
S-(2,3,4,6-tetra-O-acetyl--β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-1-S-acetyl-1,6-dithio-β-D-glucopyranose (35)



35

The trisaccharide **31** (2.92 g, 2.88 mmol) was reacted as described for the conversion of **21** to **30** to give target compound **35** (1.67 g, 57%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.28-5.17 (m, 3H, H-1, H-3, H-3''), 5.12-5.04 (m, 3H, H-3', H-2, H-4''), 4.98 (t, 1H, J = 10.0 Hz, H-2''), 4.93-4.88 (m, 3H, H-2', H-4, H-4''), 4.73 (d, 1H, J = 10.0 Hz, H-1'), 4.67(d, 1H, J = 10.0 Hz, H-1''), 4.24 (dd, 1H, J = 5.0, 13.0 Hz, H-6a''), 4.13 (dd, 1H, J = 2.0, 13.0 Hz, H-6b''), 3.86 (dt, 1H, J = 3.0, 8.5 Hz, H-5), 3.72 (ddd, 1H, J = 3.0, 5.0, 10.0 Hz, H-5''), 3.60 (dt, 1H, J = 3.0, 8.5 Hz, H-5'), 2.85-2.72 (m, 4H, H-6a, H-6b, H-6a', H-6b'), 2.41 (s, 3H, $SCOCH_3$), 2.09, 2.07, 2.06, 2.04, 2.00, 1.99, 1.98, 1.98, 1.97 (10s, 30H, $COCH_3$). HRMS (ESI) Calc. for $C_{40}H_{54}O_{24}S_3Na$ [M+Na$^+$]: 1037.2065, observed 1037.2068.

2-(Trimethylsilyl)ethyl S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranoside (36)

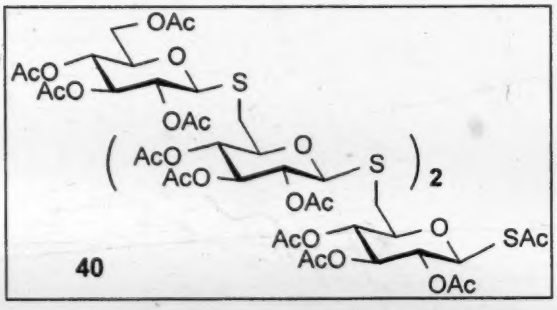


36

A mixture of trisaccharide thioacetate **35** (1.348 g, 1.33 mmol) and 6-iodo monosaccharide **18** (0.618 g, 1.20 mmol) in dry DMF (10 mL) was stirred at –20 °C for 30 min. Diethylamine (1 mL, 9.67 mmol) was then added under Ar atmosphere. The reaction mixture was stirred for a further 1 h below 0 °C then allowed to reach room temperature. Stirring was continued for 24 h, followed by removal of diethylamine in *vacuo*. The residue was diluted with CH_2Cl_2 (30 mL), washed with H_2O (2 × 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Column chromatography (hexane/ethyl acetate: 1:2) gave linear tetrasaccharide **36** (1.41 g, 78%). $[\alpha]_D$ + 3.40° (c 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23-5.13 (m, 4H), 5.08 (t, 1H, J = 10.0 Hz, H-4'''), 5.01-4.88 (m, 7H), 4.78 (d, 1H, J = 10.0 Hz), 4.60 (d, 1H, J = 10.0 Hz), 4.59 (d, 1H, J = 10.0 Hz), 4.57 (d, 1H, J = 8.0 Hz, H-1), 4.28 (dd, 1H, J = 5.5, 12.5 Hz, H-6a'''), 4.15 (dd, 1H, J = 2.5, 12.5 Hz, H-6b'''), 3.97 (dt, 1H, J = 6.0, 10.0 Hz, OC$\underline{H}_2$CH$_2$SiMe$_3$), 3.81-3.66 (m, 4H, H-5''', H-5, H-5', H-5''), 3.58 (dt, 1H, J = 7.0, 10.0 Hz, OC$\underline{H}_2$CH$_2$SiMe$_3$), 2.94-2.72 (m, 6H, H-6a'', H-6b'', H-6a', H-6b', H-6a, H-6b), 2.13-1.96 (11s, 3H each, $COCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.47, 170.05, 169.93, 169.77, 169.54, 169.50, 169.41, 169.29, 169.19, 169.17, 169.10, 169.09, 100.03, 84.05, 83.92, 83.58, 77.77, 77.42, 76.37, 73.92, 73.74, 73.65, 73.59, 72.99, 71.86, 71.71, 71.67, 71.63, 70.47, 70.04, 69.83, 68.34, 67.62, 62.32, 32.32, 31.77, 31.66, 20.91, 20.84, 20.79,

20.77, 20.76, 20.70, 20.67, 20.66, 17.95, -1.23. Anal. Calcd. for $C_{55}H_{80}O_{31}S_3Si$: C, 48.52; H, 5.92; S, 7.07; Found: C, 48.60; H, 6.02; S, 7.13.

*S-(2,3,4,6-tetra-O-acetyl--β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-2,3,4-tri-O-acetyl-1-S-acetyl-1,6-dithio-β-D-glucopyranose (**40**)*

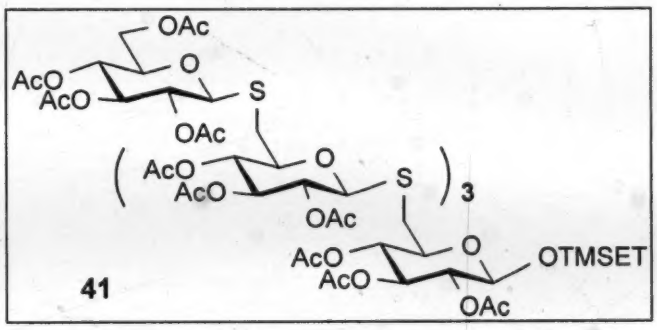


40

The TMSET tetrasaccharide **36** (8.54 g, 6.27mmol) was reacted as described for the conversion of **21** to **30** to give target compound **40** (2.73 g, 33%). $[\alpha]_D$ + 4.05° (c 1.3, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 5.29 (d, 1H, J = 10.0 Hz, H-1), 5.23 (t, 1H, J = 9.5 Hz), 5.20 (t, 1H, J = 9.5 Hz), 5.16 (t, 1H, J = 9.5 Hz), 5.12 (t, 1H, J = 9.5 Hz), 5.08 (dd, 1H, J = 9.5 Hz, 10.0 Hz), 5.07 (t, 1H, J = 9.5 Hz, H-4'''), 4.98 (t, 1H, J = 10.0 Hz), 4.96-4.87 (m, 5H), 4.82 (d, 1H, J = 10.0 Hz), 4.66 (d, 1H, J = 10.0 Hz), 4.65 (d, 1H, J = 10.0 Hz), 4.27 (dd, 1H, J = 5.0, 13.0 Hz, H-6a'''), 4.14 (dd, 1H, J = 2.0, 13.0 Hz, H-6b'''), 3.86 (dt, 1H, J = 3.0, 10.0 Hz), 3.74 (dddd, 1H, J = 1.0, 3.0, 5.0, 10.0 Hz, H-5'''), 3.72-3.63 (m, 2H), 2.90-2.75 (m, 6H), 2.40 (s, 3H, $SCOCH_3$), 2.11-1.96 (m, 39H, $COCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.74, 170.58, 170.02, 170.01, 170.00, 169.91, 169.57, 169.55, 169.53, 169.50, 169.42, 169.32, 169.28, 83.58, 83.38, 83.01, 79.98, 79.74, 78.01, 77.73, 76.12, 73.79, 73.73, 73.69, 73.65, 73.53, 71.58, 71.30, 70.10, 69.99, 69.96, 69.12, 68.30, 62.22, 31.45,

31.29, 30.76, 30.49, 20.77, 20.73, 20.72, 20.69, 20.68, 20.64, 20.61, 20.54. ESI-MS Calc. for $C_{52}H_{70}O_{31}S_4SiNa$ [M+Na^+] 1341.3, observed 1341.3.

2-(Trimethylsilyl)ethyl S-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranoside ***(41)***

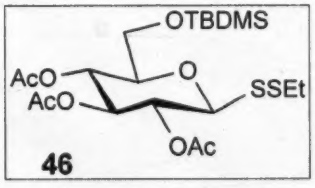


41

A mixture of tetrasaccharide thioacetate **40** (2.41 g, 1.83 mmol) and 6-iodo monosaccharide **18** (2.41 g, 1.83 mmol) in dry DMF (15 mL) was stirred at –20 °C for 30 min. Diethylamine (1.5 mL, 12.81 mmol) was then added under Ar atmosphere. The reaction mixture was stirred for a further 1 h below 0 °C then allowed to reach room temperature. Stirring was continued for 24 h, followed by removal of diethylamine in *vacuo*. The residue was diluted with CH_2Cl_2 (200 mL), washed with H_2O (2 × 100 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Column chromatography (hexane/ethyl acetate: 1:3) gave linear tetrasaccharide **41** (2.19 g, 72%). $[\alpha]_D$ +18.1° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.24 (t, 1H, J = 10.0Hz, H-3''''), 5.21 (t, 1H, J = 10.0 Hz), 5.20 (t, 1H, J =10.0 Hz), 5.16 (t, 1H, J = 10.0 Hz), 5.10 (t, 1H, J = 10.0 Hz, H-4''''), 5.05-4.91 (m, 9H), 4.89 (d, 1H, J = 10.0 Hz), 4.86 (d, 1H, J = 10.0 Hz), 4.63 (d, 1H, J = 8.0 Hz, H-1), 4.58 (d, 1H, J = 10.0 Hz),

4.57 (d, 1H, J = 10.0 Hz), 4.34 (dd, 1H, J = 10.0, 6.0 Hz, H-6a''''), 4.16 (dd, 1H, J = 12.0, 2.0 Hz, H-6b''''), 4.04 (dt, 1H, J = 10.0, 6.5 Hz, $OC\underline{H}_2CH_2SiMe_3$), 3.92 (dt, 1H, J = 10.0, 3.0 Hz), 3.83 (dt, 1H, J = 10.0, 2.5 Hz), 3.80-3.73 (m, 2H), 3.70 (dt, 1H, J = 10.0, 2.5 Hz), 3.59 (dt, 1H, J = 10.0, 6.5 Hz, $OC\underline{H}_2CH_2SiMe_3$), 2.97 (dd, 1H, J = 13.0, 3.0 Hz), 2.95-2.89 (m, 2H), 2.87-2.79 (m, 3H), 2.75 (dd, 1H, J = 13.0, 10.0 Hz), 2.67 (dd, 1H, J = 12.0, 10.0 Hz), 2.16-1.96 (all s, 48H, $COCH_3$), 0.96-0.89 (m, 2H, $OCH_2C\underline{H}_2SiMe_3$). ESI-MS Calc. for $C_{67}H_{96}O_{38}S_4SiNa$ $[M+Na^+]$ 1687.4, observed 1687.4.

Ethyl 2,3,4-tri-O-acetyl-6-O-tert-butyldimethylsilyl-1-thio-β-D-glucopyranosyl disulfide (46)

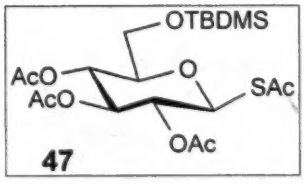


46

To a solution of compound **45** (550 mg, 1.30 mmol) in MeOH (50 mL) was added a freshly prepared methanolic solution of NaOMe (1 mM, 1 mL). After stirring for 4 h, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated. The crude residue was dissolved in pyridine (40 mL). *t*-Butyldimethylsilyl chloride (489 mg, 3.24 mmol) was added slowly to the stirred solution at 0 °C. Stirring continued for 1 h during which time the temperature was allowed to reach to rt. Acetic anhydride (15 mL) was added to the reaction mixture. The reaction solution was stirred for 24 h then diluted in CH_2Cl_2 (150 mL). The CH_2Cl_2 solution washed with dilute HCl, water, brine, dried (anhydrous Na_2SO_4), filtered, concentrated and followed by column chromatography (hexane/ethyl acetate: 2:1), which yielded compound **46** as a white solid (523 mg, 81%). $[\alpha]_D$ – 100.7° (c 1.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.27 (t, 1H, J = 9.5 Hz, H-2), 5.23 (t, 1H, J = 9.5 Hz, H-3), 5.10 (t, 1H, J = 9.5 Hz, H-4), 4.49 (d,

1H, J = 9.5 Hz, H-1), 3.72 (dd, 1H, J = 12.5, 2.5 Hz, H-6a), 3.65 (dd, J = 12.5, 5.0 Hz, H-6b), 3.56 (m, 1H, H-5), 2.94 (q, 2H, J = 7.5 Hz, $SSCH_2CH_3$), 2.03-1.97 (3s, 3H each, $COCH_3$), 1.28 (t, 3H, J = 7.5 Hz, $SSCH_2CH_3$), 0.87 (s, 9H), 0.03 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.38, 169.16, 169.13, 87.87 (C-1), 79.09, 74.44, 69.28, 68.34, 62.12 (C-6), 33.97, ($SSCH_2CH_3$), 25.78, 20.68, 20.65, 20.63 ($COCH_3$), 18.28, 14.21 ($SSCH_2CH_3$), -5.51, -5.54. HRMS (ESI): Calc. for $C_{20}H_{36}O_8S_2SiNa$ [$M+Na^+$] 519.1519, observed 519.1512.

2,3,4-tri-O-acetyl-6-O-tert-butyldimethylsilyl-1-S-acetyl-1-thio-β-D-glucopyranose (47)

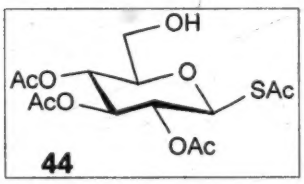


47

To a solution of compound **46** (430 mg, 0.87 mmol) in THF (50 mL) was added dithiothreitol powder (470 mg, 3.1 mmol) and 1ml Et_3N. After stirring for 6 h, the reaction mixture was concentrated under vacuo. The crude residue was dissolved in pyridine 40 mL and 25 mL acetic anhydride was added to the stirred solution at rt. The reaction solution was stirred for 24 h then diluted in CH_2Cl_2 (250 mL). The CH_2Cl_2 solution washed with dilute HCl, water, brine, dried (anhydrous Na_2SO_4), filtered, concentrated and followed by column chromatography (hexane/ethyl acetate: 2:1), which yielded compound **47** as a white solid (325 mg, 78%). ^1H NMR (300 MHz, $CDCl_3$) δ 5.29 (t, 1H, J = 9.5 Hz, H-3), 5.25 (d, 1H, J = 10.0 Hz, H-1), 5.09 (dd, 1H, J = 10.0, 9.5 Hz, H-2), 5.07 (t, 1H, J = 9.5 Hz, H-4), 3.72 (d, 1H, J = 12.0 Hz, 5.0, H-6a), 3.65 (ddd, 1H, J = 3.0, 5.5, 9.5Hz, H-5), 3.53 (dd, 1H, J = 12.0, 6.5 Hz, H-6b), 2.39 (s, 3H, $SCOCH_3$), 2.03-1.97 (3s, 9H, $COCH_3$), 0.84 (s, 9H), -0.3 (s, 6H) . ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.64 ($SCOCH_3$), 170.17,

169.92, 169.14 ($COCH_3$), 82.38 (C-1), 74.00, 72.91, 71.60, 68.8, 67.52 (C-6), 30.95 ($SCO\underline{CH_3}$), 25.84, 20.92, 20.83, 20.72 ($COCH_3$), 18.32, -5.33. HRMS (ESI): Calc. for $C_{20}H_{34}O_9SSiNa$ [$M+Na^+$] 501.1590, observed 501.1594.

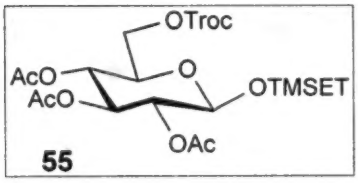
2,3,4-tri-O-acetyl -1-S-acetyl-1-thio-β-D-glucopyranose ***(44)***



44

The compound **47** (350 mg, 0.73 mmol) was stirred in 80% aqueous acetic acid (80 mL) at 70 °C for 2.5 h. The reaction solution was then co-evaporated with toluene to remove water and acetic acid, concentrated and subjected to column chromatography (hexane/ethyl acetate: 3:2) to give **44** (364 mg, 76%) as a clear syrup. $[\alpha]_D$ 13.0° (c 1.4, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.32 (t, 1H, J = 9.5 Hz, H-3), 5.27 (d, 1H, J = 10.0 Hz, H-1), 5.10 (dd, 1H, J = 10.0, 9.5 Hz, H-2), 5.07 (t, 1H, J = 9.5 Hz, H-4), 3.74 (d, 1H, J = 12.0 Hz, H-6a), 3.65 (m, 1H, H-5), 3.53 (dd, 1H, J = 12.0, 4.0 Hz, H-6b), 2.38 (s, 3H, $SCO\underline{CH_3}$), 2.06, 2.02 2.01 (3s, 9H, $COC\underline{H_3}$), ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.17, 170.15, 170.02, 169.33 ($COCH_3$), 80.14 (C-1), 78.72, 73.79, 69.21, 68.39, 61.16 (C-6), 30.83, 20.61, 20.57, 20.58 ($COCH_3$). HRMS (ESI): Calc. for $C_{14}H_{24}O_9Na$ [$M+Na^+$] 387.0726, observed 387.0723.

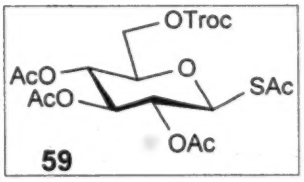
*2-(Trimethylsilyl)ethyl 2,3,4-tri-O-acetyl-6-O-trichloroethoxycarbonyl-β-D-glucopyranoside (**55**)*



55

To a solution of **17** (750 mg, 1.85 mmol) and pyridine (439 μL, 5.54 mmol) in CH_2Cl_2 (100 mL) were added 2, 2, 2- trichloroethoxycarbonyl chloride (TrocCl) (760 μL, 5.54 mmol) and DMAP (37 mg, 0.02 mmol) at 0 °C. After the solution was stirred for 1 h, excess TrocCl was distroyed with MeOH (1 mL). The mixture was concentrated and the residue was dissolved in CH_2Cl_2 (300 mL). The organic solution was washed with 1N HCl, saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel chromatography (hexane/ethyl acetate: 2:1), which gave **55** (1.00 g, 93%) as a crystalline solid. $[\alpha]_D$ – 17.6° (c 0.9, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.18 (t, 1H, J = 9.5 Hz, H-3), 5.01 (t, 1H, J = 9.5 Hz, H-4), 4.94 (dd, 1H, J = 9.5, 8.0 Hz, H-2), 4.76 (d, 1H, J = 12.0 Hz, $OCOOC\underline{H_2}Cl_3$), 4.70 (d, 1H, J = 12.0 Hz, $OCOOC\underline{H_2}Cl_3$), 4.50 (d, 1H, J = 8.0 Hz, H-1), 4.36 (dd, 1H, J = 6.0, 12.0 Hz, H-6a), 4.24 (dd, 1H, J = 12.0, 3.0 Hz, H-6b), 3.94 (dt, 1H, J = 10.0, 6.0 Hz, $O\underline{CH_2}CH_2SiMe_3$), 3.50 (ddd, 1H, J = 9.5, 5.5, 3.0 Hz, H-5), 3.54 (dt, 1H, J = 10.0, 6.5 Hz, $OC\underline{H_2}CH_2SiMe_3$), 2.03, 2.00, 1.98 (3s, 9H, $COCH_3$), 0.90-0.80 (m, 2H, $OCH_2C\underline{H_2}SiMe_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.20, 169.53, 169.17 ($COCH_3$), 150.67, 100.11 (C-1), 92.95, 76.97, 72.77, 71.38, 71.32, 69.02, 67.56, 67.08, 20.66, 20.56, 17.90, -1.43. HRMS (ESI): Calc. for $C_{20}H_{31}Cl_3O_{11}SiNa$ [M+Na^+] 603.0599, observed 603.0590.

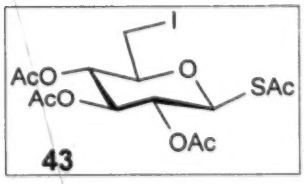
2,3,4-tri-O-acetyl-6-O-trichloroethoxycarbonyl-1-S-acetyl-1-thio-β-D-glucopyranose (59)



59

Compound **56** (850 mg, 1.46 mmol) was reacted as described for the conversion of **21** to **30** to give **59** (544 mg, 68%). Column chromatography (hexane/ethyl acetate: 3:1). $[\alpha]_D$ – 3.6° (c 0.4, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.29 (t, 1H, J = 9.5 Hz, H-3), 5.27 (d, 1H, J = 10.0 Hz, H-1), 5.13 (dd, 1H, J = 10.0, 9.5 Hz, H-2), 5.10 (t, 1H, J = 9.5 Hz, H-4), 4.79 (d, 1H, J = 12.0 Hz, OCOOC$\underline{H_2}Cl_3$), 4.73 (d, 1H, J = 12.0 Hz, OCOOC$\underline{H_2}Cl_3$), 4.38 (dd, 1H, J = 12.0 Hz, 5.0, H-6a), 4.27 (dd, 1H, J = 12.0, 6.5 Hz, H-6b), 3.91 (ddd, 1H, J = 9.5, 5.5, 3.0 Hz, H-5), 2.40 (s, 3H, SCO$\underline{CH_3}$), 2.06, 2.02, 2.01 (3s, 9H, $COCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.84, 169.99, 169.48, 169.25, 153.66, 92.95, 80.19 (C-1), 76.99, 75.82, 73.75, 68.95, 68.34, 66.47, 30.82, 20.56; HRMS (ESI): Calc. for $C_{17}H_{21}Cl_3O_{11}SNa$ [M+Na^+] 560.9768, observed 560.9760.

2,3,4-tri-O-acetyl 6-O-deoxy-6-iodo-1-S-acetyl-1-thio-β-D-glucopyranose (43)

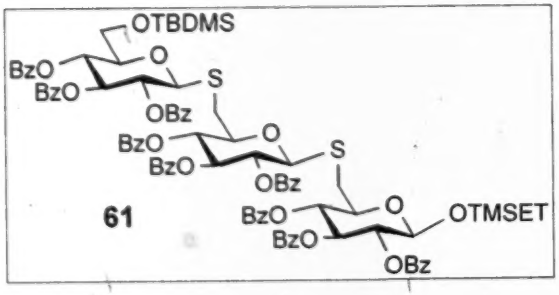


43

A mixture of disaccharide **44** (200 mg, 0.55 mmol), triphenylphosphine (1.44 g, 5.5 mmol), and carbon tetraiodide (1.43 g, 2.75 mmol), in dry toluene (30 mL), was vigorously stirred at 60 °C for 10 min. The reaction mixture was filtered through a celite pad then concentrated in *vacuo*. The residue was purified by silica gel chromatography

(toluene/ethyl acetate: 10:1), which gave **43** (216 mg, 83%) as a crystalline solid. $[\alpha]_D$ – 10.0 ° (c 0.9, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.30 (t, 1H, J = 10.0 Hz, H-1), 5.28 (d, 1H, J = 9.5 Hz, H-3), 5.12 (dd, 1H, J = 10.0, 9.5 Hz, H-2), 4.96 (t, 1H, J = 9.5 Hz, H-4), 3.55 (ddd, 1H, J = 9.5, 5.5, 3.5 Hz, H-5), 3.32 (dd, 1H, J = 12.0, 3.0 Hz, H-6a), 3.14 (dd, 1H, J = 12.0, 6.5 Hz, H-6b), 2.40 (s, 3H, $SCOCH_3$), 2.04, 2.02, 2.00 (3s, 9H, $COCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.86, 170.01, 169.32, 169.27, 80.02, 73.58, 72.09, 69.10, 68.80, 30.84, 20.68, 20.57, 20.56, 2.61. HRMS (ESI): Calc. for $C_{14}H_{19}IO_8SNa$ $[M+Na^+]$ 496.9743, observed 496.9736.

2-(Trimethylsilyl)ethyl S-(2,3,4-tri-O-benzoyl-6-O-tert-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranoside ***(61)***



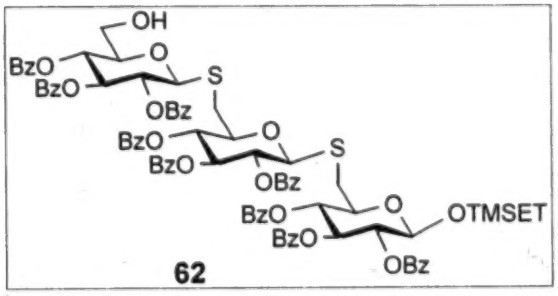
To a solution of compound **31** (336 mg, 0.318 mmol) in MeOH (10 mL) was added a freshly prepared methanolic solution of NaOMe (1 mM, 1 mL). After stirring for 5 h, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated, affording trisaccharide **60** (202 mg, quant.). ^1H NMR (500 MHz, $CDCl_3$) δ 4.65 (d, 1H, J = 10.0 Hz), 4.62 (d, 1H, J = 10.0 Hz), 4.57 (d, 1H, J = 10.0 Hz, H-1), 4.02 (dt, 1H, J = 6.0, 10.0 Hz, $OCH_2CH_2SiMe_3$), 3.91 (dd, 1H, J = 1.0, 12.5 Hz), 3.81 (dt, 1H, J = 6.5, 10.0 Hz, $OCH_2CH_2SiMe_3$), 3.76-3.69 (m, 2H), 3.62 (dt, 1H, J = 2.0, 9.0 Hz), 3.52-3.40 (m, 5H),

3.39-3.22 (m, 10H), 2.90-2.80 (m, 2H), 1.04 (m, 2H, $OCH_2C\underline{H_2}SiMe_3$), 0.00 (s, 9H, $OCH_2CH_2Si\underline{Me_3}$). Anal. Calcd. for $C_{23}H_{44}O_{14}S_2Si$: C, 43.38; H, 6.96; S, 10.07; Found: C, 43.25; H, 6.96; S, 9.85.

Crude residue **60** was dissolved in pyridine (10 mL). *t*-Butyldimethylsilyl chloride (72 mg, 0.447 mmol) was added slowly to the stirred solution at 0 °C. Stirring continued for 4h during which time the temperature was allowed to reach to rt. After compound **60** had been completely consumed (TLC, dichloromethane/methanol: 5:1), benzoyl chloride (554 μL, 4.8 mmol) was added to the reaction mixture. The reaction was stirred for 24 h then diluted in CH_2Cl_2 (30 mL). The CH_2Cl_2 solution washed with dilute HCl, water, brine, dried (anhydrous Na_2SO_4), filtered, concentrated and followed by column chromatography (toluene/ethyl acetate: 10:1), which yielded **61** as a white solid (530 mg, 78%). $[\alpha]_D$ +2.86° (c 1.0, $CHCl_3$); 5.81 (t, 1H, J = 9.5 Hz, H-3''), 5.77 (t, 1H, J = 9.5 Hz, H-3'), 5.47 (t, 1H, J = 10.0 Hz, H-4''), 5.46-5.34 (m, 5H, H-2, H-2', H-2'', H-4', H-4), 4.82 (d, 1H, J = 10.0 Hz, H-1'), 4.81 (d, 1H, J = 8.0 Hz, H-1), 4.61 (d, 1H, J = 10.0.0 Hz, H-1''), 4.05 (dt, 1H, J = 3.0, 9.0 Hz, H-5), 4.61 (d, 1H, J = 10.0 Hz, H-1''), 4.01 (dt, 1H, J = 6.0, 10.0 Hz, $OC\underline{H_2}CH_2SiMe_3$), 3.91 (dt, 1H, J = 3.0, 10.0 Hz, H-5'), 3.79-3.74 (m, 2H, H-5'', H-6a'), 3.70 (dd, 1H, J = 5.5, 12.0 Hz, H-6b''), 3.57 (dt, 1H, J = 6.5, 10.0 Hz, $OC\underline{H_2}CH_2SiMe_3$), 3.04 (dd, 1H, J = 3.5, 14.0 Hz, H-6a), 3.00 (dd, 1H, J = 3.0, 14.0 Hz, H-6a'), 2.93 (dd, 1H, J = 9.0, 14.0 Hz, H-6b), 2.89 (dd, 1H, J = 9.0, 14.0Hz, H-6b'), 0.96-0.89 (m, 2H, $OCH_2C\underline{H_2}SiMe_3$), 0.80 (s, 9H, t-butyl), 0.00 (s, 6H, t-butyl), -0.20 (s, 9H,$OCH_2CH_2SiMe_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.66, 165.64, 165.51, 165.30, 165.15, 165.00, 164.99, 164.93, 164.82, 133.38, 133.30, 133.18, 133.17,

133.05, 133.04, 132.94, 132.93, 132.84, 129.98, 129.84, 129.83, 129.80, 129.73, 129.68, 129.67, 129.63, 129.54, 129.53, 129.23, 129.22, 129.20, 129.19, 129.11, 128.98, 128.86, 128.46, 128.40, 128.36, 128.27, 128.22, 128.16, 128.13, 128.11, 100.45, 84.33, 83.32, 79.62, 78.35, 74.64, 74.47, 74.12, 73.11, 72.75, 72.30, 72.15, 71.15, 70.88, 69.42, 67.65, 32.44, 31.33, 25.93, 18.42, 17.94, -1.26. Anal. Calcd. for $C_{92}H_{94}O_{23}S_2Si_2$: C, 65.46; H, 5.61; S, 3.80; Found: C, 65.16; H, 5.59; S, 4.01.

*2-(Trimethylsilyl)ethyl S-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoy-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O- benzoyl-6-thio-β-D-glucopyranoside (**62**)*

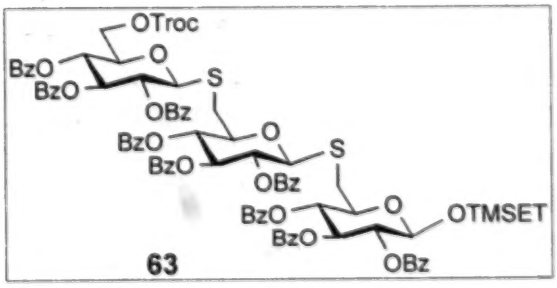


62

The compound **61** (492 mg, 0.291 mmol) was stirred in 80% aqueous acetic acid (25 mL) at 80 °C for 2.5 h. The solution was then co-evaporated with toluene to remove water and acetic acid, concentrated and subjected to column chromatography (hexane/ethyl acetate: 3:2) to give **62** (372 mg, 81%) as a clear syrup. $[\alpha]_D$ + 13.75° (c 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.90-7.20 (m, 45H, aromatic H), 5.88 (t, 1H, J = 9.5 Hz, H-3), 5.85 (t, 1H, J = 9.5 Hz, H-3''), 5.76 (t, 1H, J = 9.5 Hz, H-3'), 5.53 (t, 1H, J = 10.0 Hz, H-4'), 5.48-5.42 (m, 2H, H-2, H-2'), 5.37 (t, 1H, J = 10.0 Hz, H-4), 5.36 (t, 1H, J = 10.0 Hz, H-4''), 4.09 (dt, 1H, J = 2.0, 9.0 Hz, H-5'), 4.01 (m, 1H, H-5'), 3.98 (m, 1H, $OC\underline{H_2}CH_2SiMe_3$), 3.77 (dddd, 1H, J = 2.0, 5.0, 10.0 Hz, H-5'), 3.73 (m, 1H, H-6a''), 3.55

(dt, 1H, J = 6.5, 10.0 Hz, $OC\underline{H_2}CH_2SiMe_3$), 3.50 (m, 1H, H-6b''), 3.13 (dd, 1H, J = 3.0, 14.0 Hz, H-6a'), 3.05 (dd, 1H, J = 3.0, 14.0 Hz, H-6a), 2.97 (dd, 1H, J = 9.0, 14.0 Hz, H-6b), 2.94 (dd, 1H, J = 7.0, 14.0 Hz, H-6b'), 0.96-0.89 (m, 2H, $OCH_2C\underline{H_2}SiMe_3$), -0.20 (s, 9H, $OSiMe_2C\underline{Me_3}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.71, 165.55, 165.54, 165.40, 165.37, 165.00, 164.99, 164.94, 133.52, 133.43, 133.22, 133.20, 133.09, 133.06, 132.98, 132.88, 129.94, 129.86, 129.80, 129.79, 129.78, 129.71, 129.68, 129.64, 129.63, 129.53, 129.08, 129.07, 129.06, 128.97, 128.97, 128.93, 128.84, 128.80, 128.74,128.63, 128.48, 128.46, 128.34, 128.30, 128.19, 128.18, 128.14, 100.46, 83.96, 82.82, 79.29, 77.87, 74.77, 74.18, 74.08, 73.15, 72.79, 72.16, 71.49, 70.94, 70.78, 69.43, 67.69, 61.92, 31.88, 30.49, 17.92, -1.26. Anal. Calcd. for $C_{86}H_{80}O_{23}S_2Si$: C, 65.63; H, 5.12; S, 4.08. Found: C, 65.24; H, 5.20; S, 4.10.

*2-(Trimethylsilyl)ethyl S-(2,3,4-tri-O-benzoyl-6-O-trichloroethoxycarbonyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranoside (**63**)*

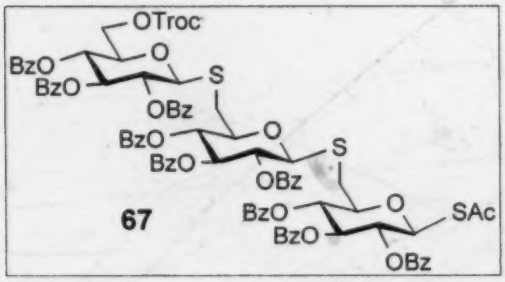


63

To a solution of **62** (348 mg, 0.221 mmol) and pyridine (54 μL, 0.663 mmol) in CH_2Cl_2 (10 mL) were added 2, 2, 2- trichloroethoxycarbonyl chloride (TrocCl) (91 μL, 0.66 mmol) and DMAP (7 mg, 0.044 mmol) at 0 °C. After the solution was stirred for 1 h, excess TrocCl was destroyed with MeOH (1 mL). The mixture was concentrated and the residue was

dissolved in CH_2Cl_2 (20 mL). The organic solution was washed with 1N HCl, saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel chromatography (hexane/ethyl acetate: 2:1), which gave **63** (360 mg, 93%) as a crystalline solid. $[\alpha]_D$ + 10.3° (c 1.1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 8.90-7.20 (m, 45H, aromatic H), 5.90 (t, 1H, J = 10.0 Hz, H-3), 5.88 (t, 1H, J = 10.0 Hz, H-3''), 5.77 (t, 1H, J = 9.5 Hz, H-3'), 5.53 (t, 1H, J = 10.0 Hz, H-4''), 5.50-5.41 (m, 4H, H-2, H-2'', H-2', H-4), 5.37 (t, 1H, J = 10.0 Hz, H-4'), 4.88 (d, 1H, J = 10.0 Hz, H-1'), 4.85 (d, 1H, J = 8.0 Hz, H-1), 4.80 (d, 1H, J = 12.0 Hz, OCOOC$\underline{H_2}Cl_3$), 4.76 (d, 1H, J = 12.0 Hz, OCOOC$\underline{H_2}Cl_3$), 4.77 (d, 1H, J = 10.0.0 Hz, H-1''), 4.40 (dd, 1H, J = 3.0, 12.0 Hz, H-6a''), 4.37 (dd, 1H, J = 6.0, 12.0 Hz, H-6b''), 4.10 (dt, 1H, J = 3.0, 9.0 Hz, H-5), 4.06 (m, 1H, H-5''), 4.02 (dt, 1H, J = 6.0, 10.0 Hz, OC$\underline{H_2}CH_2SiMe_3$), 3.96 (dt, 1H, J = 3.0, 9.0 Hz, H-5'), 3.59 (dt, 1H, J = 7.0, 9.0 Hz, OC$\underline{H_2}CH_2SiMe_3$), 3.12 (dd, 1H, J = 3.0, 14.0 Hz, H-6a), 3.01 (dd, 1H, J = 9.0, 14.0 Hz, H-6b), 2.97 (dd, 1H, J = 3.0, 14.0 Hz, H-6a'), 2.91 (dd, 1H, J = 9.0, 14.0 Hz, H-6b'), 0.96-0.89 (m, 2H, $OCH_2CH_2Si\underline{Me_3}$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.69, 165.55, 169.50, 165.44, 165.12, 165.04, 165.02, 164.94, 164.90, 153.69, 133.38, 133.34, 133.21, 133.19, 133.08, 133.06, 132.94, 132.84, 129.95, 129.86, 129.81, 129.79, 129.78, 129.73, 129.69, 129.54, 129.12, 129.05, 129.00, 128.94, 128.86, 128.85, 128.76, 128.65, 128.50, 128.41, 128.35, 128.29, 128.18, 128.11, 100.46, 94.37, 84.39, 84.04, 78.43, 76.89, 76.09, 74.62, 74.08, 73.91, 73.09, 72.81, 72.22, 72.17, 71.08, 70.76, 69.32, 67.69, 67.17, 32.52, 31.98, 17.93, -1.26. Anal. Calcd. for $C_{89}H_{81}Cl_3O_{25}S_2Si$: C, 61.11; H, 4.67; Cl, 6.08; S, 3.67. Found: C, 60.93; H, 4.58; S, 3.77.

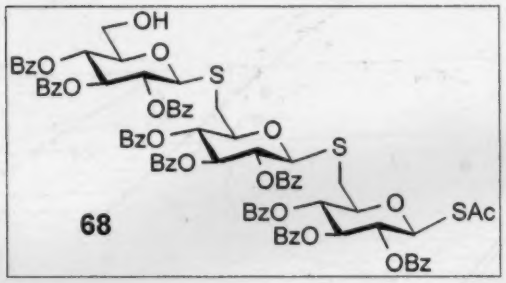
S-(2,3,4-tri-O-benzoyl-6-O-trichloroethoxycarbonyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-1-S-acetyl-1,6-dithiol-β-D-glucopyranose (67)



67

Compound **67** (324 mg, 0.185 mmol) was reacted as described for the conversion of **21** to **30** to give **67** (224 mg, 71%). Column chromatography (toluene/ethyl acetate: 15:1) $[\alpha]_D$ + 27.7° (c 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.99-7.12 (m, 45H, aromatic H), 5.94 (t, 1H, J = 9.0 Hz, H-3''), 5.91 (t, 1H, J = 9.0 Hz, H-3), 5.65-5.52 (m, 4H, H-2, H-4'', H-4, H-2''), 5.48 (t, 1H, J = 10.0 Hz, H-2'), 5.43 (t, 1H, J = 9.5 Hz, H-4'), 5.00 (d, 1H, J = 10.0 Hz, H-1'), 4.95 (d, 1H, J = 10.0 Hz, H-1''), 4.79 (d, 1H, J = 12.0 Hz, $OCOOCH_2Cl_3$), 4.75 (d, 1H, J = 12.0 Hz, $OCOOCH_2Cl_3$), 4.46-4.40 (m, 2H, H-6a'', H-6b''), 4.20 (dt, 1H, J = 3.0,10.0 Hz, H-5), 4.13 (dt, 1H, J = 3.5, 10.0 Hz, H-5''), 3.90 (m, 1H, H-5'), 3.07 (dd, 1H, J = 8.0, 15.0 Hz, H-6a), 3.00-2.97 (m, 3H, H-6b, H-6a', H-6b'), 2.38 (s, 3H, $SCOCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.12 ($SCOCH_3$), 165.59, 165.57, 165.51, 165.21, 165.08, 164.99, 164.92, 153.70, 133.37, 133.30, 133.18, 133.14, 133.06, 133.02, 129.93, 129.89, 129.86, 129.78, 129.76, 129.71, 129.66, 129.64, 129.18, 129.18, 128.94, 128.90, 128.81, 128.71, 128.42, 128.36, 128.36, 128.31, 128.27, 128.18, 128.16, 128.15, 94.37, 84.01, 83.26, 80.47, 80.18, 78.64, 76.92, 75.98, 74.18, 74.10, 73.97, 72.17, 71.87, 71.04, 70.95, 70.06, 69.38, 67.06, 31.80, 31.27, 30.97 ($SCOCH_3$). Anal. Calcd. for $C_{86}H_{71}Cl_3O_{25}S_3$: C, 60.51; H, 4.19; Cl, 6.23; S, 5.64. Found: C, 60.67; H, 4.28; S, 5.59.

S-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O- benzoyl-1-S-acetyl-1, 6-dithiol-β-D-glucopyranose (68)

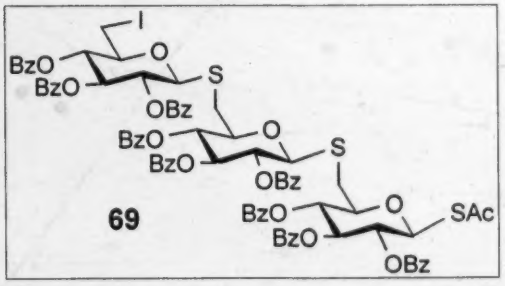


68

To a solution of **67** (187 mg, 0.109 mmol) in acetic acid (8 mL) was added Zn powder (610 mg, 10.9 mmol), and the mixture was stirred at room temperature for 24 h and then filtered, concentrated. A solution of the residue in CH_2Cl_2 (20 mL) was washed with water, saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel chromatography (toluene/ethyl acetate: 10:1) to give **68** (143 mg, 85%) as a clear syrup. $[\alpha]_D$ +5.2° (c 1.1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.97-7.20 (m, 45H, aromatic H), 5.92, 5.91 (2t, 2H, J = 9.0 Hz, H-3'', H-3), 5.74 (t, 1H, J = 9.0 Hz, H-3'), 5.65 (t, 1H, J = 9.0 Hz, H-2), 5.60 (d, 1H, J = 10.0 Hz, H-1), 5.57 (t, 1H, J = 10.0 Hz, H-4'), 5.53-5.47 (m, 3H, H-4, H-2', H-2''), 5.41 (t, 1H, J = 10.0 Hz, H-4''), 4.42 (dt, 1H, J = 3.0, 8.0 Hz, H-5), 3.90 (ddd, 1H, J = 3.0, 6.5, 10.0 Hz, H-5'), 3.85 (ddd, 1H, J = 2.0, 5.5, 10.0 Hz, H-5''), 3.76 (m, 1H, H-6a''), 3.5 (m, 1H, H-6b''), 3.17 (dd, 1H, J = 3.0, 14.0 Hz, H-6a'), 3.07-3.00 (m, 2H, H-6a, H-6b'), 2.09 (dd, 1H, J = 2.0, 15.0 Hz, H-6b), 2.38 (s, 3H, $SCOCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.31, 165.63, 165.61, 165.57, 165.52, 165.42, 165.19, 165.09, 165.06, 164.96, 133.45, 133.40, 133.38, 133.32, 133.17, 133.15, 133.05, 133.04, 129.95, 129.91, 129.86, 129.81, 129.75, 129.66, 129.16, 128.89, 128.87, 128.8, 128.85, 128.81, 128.80, 128.79, 128.75, 128.75, 128.68, 128.43, 128.41, 128.34, 128.33, 128.28,

128.17, 128.17, 82.93, 80.51, 80.46, 79.21, 77.98, 77.98, 74.29, 74.14, 71.83, 71.71, 70.94, 70.07, 69.48, 31.00. Anal. Calcd. for $C_{83}H_{70}O_{23}S_3$: C, 65.09; H, 4.61; S, 6.28. Found: C, 64.84; H, 4.66; S, 6.27.

*S-(2,3,4-tri-O-benzoyl-6-O-deoxy-6-iodo-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-1-S-acetyl-1,6-dithiol-β-D-glucopyranose (**69**)*

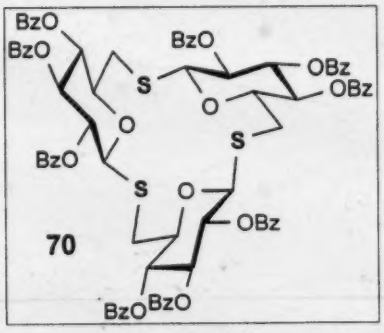


69

A mixture of trisaccharide **68** (115 mg, 0.075 mmol), triphenylphosphine (197 mg, 0.75 mmol), and carbon tetraiodide (195 mg, 0.375 mmol), in dry toluene (10 mL), was vigorously stirred at 60 °C for 10 min. The reaction mixture was filtered through a celite pad then concentrated in *vacuo*. The residue was purified by silica gel chromatography (toluene/ethyl acetate: 10:1), which gave **69** (110 mg, 89%) as a crystalline solid. $[\alpha]_D$ + 32.16° (c 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.98-7.16 (m, 45H, aromatic H), 5.94 (t, 1H, J = 9.0 Hz, H-3), 5.90 (t, 1H, J = 9.5 Hz, H-3''), 5.80 (t, 1H, J = 9.0 Hz, H-3'), 5.68 (t, 1H, J = 10.0 Hz, H-2), 5.63 (d, 1H, J = 10.0 Hz, H-1), 5.55 (t, 1H, J = 10.0 Hz, H-2''), 5.55 (t, 1H, J = 10.0 Hz, H-2''), 5.52 (t, 1H, J = 9.5 Hz, H-4), 5.46 (2t, 2H, J = 9.0 Hz, H-4'', H-2'), 5.38 (t, 1H, J = 10.0 Hz, H-4'), 5.08 (d, 1H, J = 10.0 Hz, H-1'), 4.98 (d, 1H, J = 10.0 Hz, H-1''), 4.21 (dt, 1H, J = 3.0, 10.0 Hz, H-5), 4.06 (dt, 1H, J = 3.0, 10.0 Hz, H-5'), 3.81 (dt, 1H, J = 3.0, 10.0 Hz, H-5''), 3.32 (dd, 1H, J = 2.0, 12.0 Hz, H-6a''), 3.22 (dd, 1H, J = 9.0, 11.0 Hz,

H-6b''), 3.13 (dd, 1H, J = 9.0, 14.0 Hz, H-6a'), 3.09 (dd, 1H, J = 9.0, 15.0 Hz, H-6a), 3.05 (dd, 1H, J = 3.0, 14.0 Hz, H-6b''), 2.90 (dd, 1H, J = 3.0, 15.0 Hz, H-6b), 2.39 (s, 3H, $SCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.16, 165.62, 165.53, 165.24, 165.16, 165.09, 164.97, 133.44, 133.38, 133.24, 133.18, 133.15, 133.04, 132.98, 129.95, 129.92, 129.89, 129.80, 129.70, 129.67, 129.62, 129.14, 129.11, 128.93, 128.85, 128.83, 128.77, 128.65, 128.43, 128.41, 128.38, 128.35, 128.28, 128.17, 128.15, 128.13, 84.47, 83.29, 80.47, 80.34, 78.75, 78.09, 74.08, 73.48, 72.66, 72.63, 72.04, 71.47, 71.28, 70.08, 32.94, 31.40, 31.06 ($SCOCH_3$), 3.61 (C6''). Anal. Calcd. for $C_{83}H_{69}IO_{22}S_3$: C, 60.73; H, 4.24; I, 7.73; S, 5.86. Found: C, 60.59; H, 4.10; S, 5.93.

Cyclo [S-(2,3,4-tri-O-benzoyl-6-thiol-β-D-glucopyranosyl)-(1→6)]$_3$ (70)

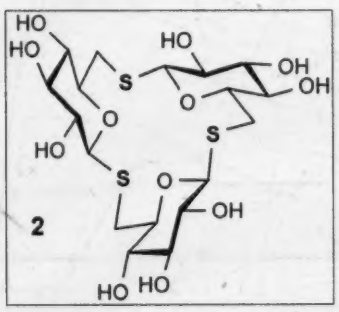


70

To a solution of linear trisaccharide **69** (82 mg, 0.05 mmol) in dry DMF (4 mL) at –20 °C was added diethylamine (40 μL, 0.39 mmol). The solution was stirred for 30 min at 0 °C then allowed to reach room temperature. After 3 h, the reaction was complete (TLC, toluene/EtOAc: 6:1) and mixture was concentrated in *vacuo*. Solution of the residue in CH_2Cl_2 (20 mL), was washed with H_2O (3 × 20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and subjected to column chromatography (toluene/EtOAc: 20: 1) to give **70** (68 mg, 92 %) as a white foam. $[\alpha]_D$ +5.4° (c 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.91-7.24 (m, 15H, aromatic H), 5.84 (t, 1H, J = 9.0 Hz, H-3), 5.46 (t, 1H, J = 9.0 Hz, H-2), 5.42 (t, 1H, J = 9.0 Hz, H-4), 5.12 (d, 1H, J = 10.0 Hz, H-1), 4.06

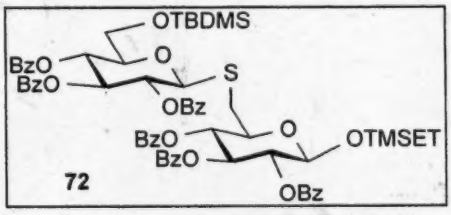
(dt, 1H, J = 1.0, 10.0 Hz, H-5), 3.18 (dd, 1H, J = 9.0, 15.0 Hz, H-6a), 2.88 (d, 1H, J = 15.0 Hz, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$) δ 133.36, 133.19, 133.13, 129.80, 129.77, 129.66, 129.11, 128.81, 128.73, 128.36, 128.29, 128.21, 83.34, 79.51, 73.96, 72.38, 71.03, 31.85. Anal. Calcd. for $C_{81}H_{66}O_{21}S_3$: C, 66.11; H, 4.52; S, 6.54; Found: C, 66.43; H, 4.89; S, 6.24.

Cyclo [S-(6-thiol-β-D-glucopyranosyl)-(1→6)]$_3$ (2)



To a solution of compound **70** (40 mg, 0.027 mmol) in and THF (1 mL) and MeOH (6 mL) was added a freshly prepared methanolic solution of NaOMe (1 mM, 0.5 mL) and stirring was continued at room temperature overnight. The reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated. Solution of the residue in H_2O (20 mL) was washed with ether (3 × 20 mL) and concentrated by co-evaporation with toluene. The crude residue was re-dissolved in H_2O (1 mL), loaded on three Waters C18 Sep-Pak cartridges and then eluted with 10 mL H_2O. The eluant containing the product was lyophilized, affording **2** as a white solid (14.5 mg, quant.). $[\alpha]_D$ + 9.7° (c 1.0, H_2O); ^{1}H NMR (600 MHz, D_2O, 27 °C) δ = 4.71 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 3.60 (t, 1H, J = 9.0 Hz, H-5), 3.44 (t, 1H, J = 9.0 Hz, H-3), 3.31 (t, 1H, J = 10.0 Hz, H-2), 3.27 (t, 1H, J = 9.0 Hz, H-4), 3.08 (d, 1H, J = 15.0 Hz, H-6a), 2.89 (dd, 1H, J = 15.0 Hz, J = 8.0 Hz, H-6b); ^{13}C NMR (125 MHz, D_2O, 27 °C) δ 86.63, 81.09, 78.06, 73.63, 72.76, 32.66. HRMS (ESI): Calc. for $C_{18}H_{30}O_{12}S_3Na$ [M+Na^+] 557.0797, observed 557.0791.

2-(Trimethylsilyl)ethyl S-(2,3,4-tri-O-benzoyl-6-O-tert-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranoside (72)

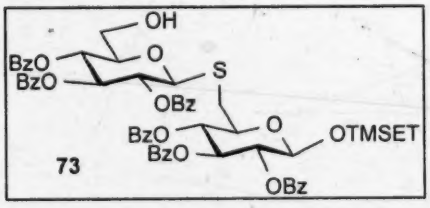


72

To a solution of compound **21** (9.12 g, 12.12 mmol) in MeOH (50 mL) was added a freshly prepared methanolic solution of NaOMe (1 mM, 1 mL). After stirring for 5 h, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated, affording TMSET disaccharide **71** (5.56 g, quant.). Crude residue **71** was dissolved in pyridine (30 mL). *t*-Butyldimethylsilyl chloride (4.58 g, 30.3 mmol) was added slowly to the stirred solution at 0 °C. Stirring continued for 4 h during which time the temperature was allowed to reach to rt. After compound **71** had been completely consumed (TLC, dichloromethane/methanol: 5:1), benzoyl chloride (10.1 mL, 87.3 mmol) was added to the reaction mixture. The reaction solution was stirred for 24 h then diluted in CH_2Cl_2 (350 mL). The CH_2Cl_2 solution washed with dilute HCl, water, brine, dried (anhydrous Na_2SO_4), filtered, concentrated and followed by column chromatography (toluene/ethyl acetate: 10:1), which yielded compound **72** as a white solid (10.8 g, 75%). $[\alpha]_D$ –3.5° (c 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.99-7.20 (m, 30H, aromatic H), 5.80 (t, 1H, J = 9.5 Hz, H-3'), 5.79 (t, 1H, J = 9.5 Hz, H-3), 5.47 (t, 1H, J = 10.0 Hz, H-4'), 5.44 (dd, 1H, J = 9.5, 8.0 Hz, H-2), 5.40 (t, 1H, J = 9.5 Hz, H-2'), 5.36 (t, 1H, J = 9.5 Hz, H-4), 4.94 (d, 1H, J = 10.0 Hz, H-1'), 4.85 (d, 1H, J = 8.0 Hz, H-1), 4.04 (dt, 1H, J = 10.0, 6.0 Hz, $OC\underline{H}_2CH_2SiMe_3$) 4.00 (dt, 1H, J = 9.0, 3.0 Hz, H-5), 3.80 – 3.76 (m, 2H, H-5',

H-6a'), 3.72 (dd, 1H, J = 12.0,6.0 Hz, H-6b'), 3.57 (dt, 1H, J = 10.0, 6.5 Hz, $OCH_2C\underline{H_2}SiMe_3$), 3.04 (dd, 1H, J = 14.0, 9.0 Hz, H-6a), 2.99 (dd, 1H, J = 14.0, 3.0 Hz, H-6b), 0.96-0.83 (m, 2H, $OCH_2C\underline{H_2}SiMe_3$), 0.80 (s, 9H), 0.00 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.75, 165.74, 165.37, 155.15, 164.98, 164.91, 133.46, 133.23, 133.19, 133.07, 133.06, 133.00, 129.84, 129.83, 129.76, 129.71, 129.69, 129.53, 129.24, 129.21, 129.24, 129.21, 128.97, 129.95, 128.20, 128.47, 128.33, 128.31, 128.21, 100.54, 83.72, 79.43, 75.05, 74.44, 73.14, 72.67, 72.05, 71.13, 69.35, 67.67, 62.71, 31.31, 25.84, 18.32, 17.80, -1.38, -5.33. Anal. Calc. For $C_{65}H_{72}O_{16}SSi_2$: C, 65.19; H, 6.06; O, 21.38; S, 2.68; Si, 4.69; Found: C, 65.10; H, 6.03; S, 2.88.

2-(Trimethylsilyl)ethyl S-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranoside (73)

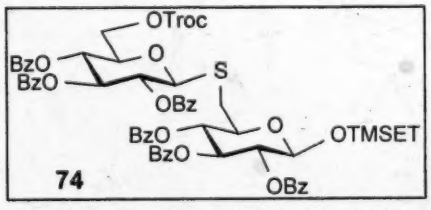


73

The compound **72** (10.4 g, 8.68 mmol) was stirred in 80% aqueous acetic acid (200 mL) at 80 °C for 2.5 h. The reaction solution was then co-evaporated with toluene to remove water and acetic acid, concentrated and subjected to column chromatography (hexane/ethyl acetate: 3:2) to give **73** (6.86 g, 79%) as a clear syrup. $[\alpha]_D$ – 22.1° (c 1.0, $CHCl_3$); 1H NMR (600 MHz, $CDCl_3$) δ 7.99-7.20 (m, 30H, aromatic H), 5.86 (t, 1H, J = 9.5 Hz, H-3'), 5.81 (t, 1H, J =9.5 Hz, H-3), 5.54 (t, 1H, J = 9.5 Hz, H-4), 5.46-5.40 (m, 2H, H-2, H-2'), 5.34 (t, 1H, J = 9.5 Hz, H-4'), 4.91 (d, 1H, J = 10.0 Hz, H-1'), 4.78 (d, 1H, J = 8.0 Hz, H-1), 4.06 (dt, 1H, J = 9.0, 3.0 Hz, H-5), 4.02 (dt, 1H, J = 10.0, 6.0 Hz, $OC\underline{H_2}CH_2SiMe_3$), 3.76 (ddd, 1H,

J = 9.0, 3.0 Hz, 1.0 Hz, H-5'), 3.74 – 3.70 (m, 1H, H-6a'), 3.58 (dt, 1H, J = 10.0, 6.5 Hz, $OCH_2C\underline{H}_2SiMe_3$), 3.52 – 3.49 (m, 1H, H-6b'), 3.12 (dd, 1H, J = 14.0, 3.0 Hz, H-6a), 3.04 (dd, 1H, J = 14.0, 7.0 Hz, H-6b), 0.96-0.83 (m, 2H, $OCH_2C\underline{H}_2SiMe_3$) ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.77, 165.75, 165.66, 165.65, 165.18, 164.98, 133.61, 133.59, 133.31, 133.19, 133.11, 133.03, 129.86, 129.82, 129.76, 129.72, 129.65, 129.49, 129.12, 128.89, 128.84, 128.80, 128.58, 128.54, 128.44, 128.40, 128.36, 128.26, 128.22, 100.51, 83.32, 79.13, 74.44, 73.97, 73.12, 71.97, 71.91, 70.86, 69.38, 67.67, 61.50, 30.58, 17.85, -1.43. Anal. Calc. For $C_{59}H_{58}O_{16}SSi$: C, 65.42; H, 5.40; O, 23.63; S, 2.96; Si, 2.59 Found: C, 65.19; H, 5.76; S, 2.97.

*2-(Trimethylsilyl)ethyl S-(2,3,4-tri-O-benzoyl-6-O-trichloroethoxycarbonyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranoside (**74**)*

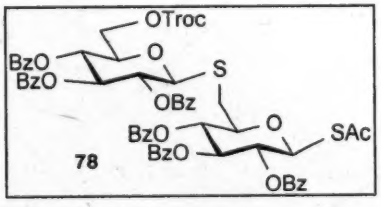


74

To a solution of **73** (6.00 g, 4.77 mmol) and pyridine (1.15 mL, 14.3 mmol) in CH_2Cl_2 (100 mL) were added 2, 2, 2- trichloroethoxycarbonyl chloride (TrocCl) (1.97 mL, 14.3 mmol) and DMAP (160 mg, 0.1 mmol) at 0 °C. After the solution was stirred for 1 h, excess TrocCl was distroyed with MeOH (10 mL). The mixture was concentrated and the residue was dissolved in CH_2Cl_2 (300 mL). The organic solution was washed with 1N HCl, saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel chromatography (hexane/ethyl acetate: 2:1), which gave **74** (5.70 g, 95%) as a crystalline solid. $[\alpha]_D$ – 0.7° (c 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.99-7.20 (m, 30H, aromatic H), 5.83 (t, 1H, J =

9.5 Hz, H-3'), 5.81 (t, 1H, J = 9.5 Hz, H-3), 5.48 (t, 1H, J = 9.5 Hz, H-4'), 5.45 (t, 1H, J = 9.5 Hz, 8.0 Hz, H-2), 5.44 (dd, 1H, J = 9.5 Hz, H-2'), 5.38 (t, 1H, J = 9.5 Hz, H-4), 4.98 (d, 1H, J = 10.0 Hz, H-1'), 4.83 (d, 1H, J = 12.0 Hz, OCOOC$\underline{H}_2Cl_3$), 4.78 (d, 1H, J = 8.0 Hz, H-1), 4.77 (d, 1H, J = 12.0 Hz, OCOOC$\underline{H}_2Cl_3$), 4.37 (dd, 1H, J = 12.0 Hz, 3.0 Hz, H-6a'), 4.36 (dd, 1H, J = 12.0 Hz, 5.0 Hz, H-6b'), 4.07 (dt, 1H, J = 9.5 Hz, J = 6.0 Hz, OC$\underline{H}_2CH_2SiMe_3$), 4.03-3.90 (m, 2H, H-5, H-5'), 3.62 (dt, 1H, J = 9.0 Hz, J = 7.0 Hz, $OCH_2C\underline{H}_2SiMe_3$), 3.04 (dd, 1H, J = 14.0 Hz, 9.0 Hz, H-6a), 2.96 (dd, 1H, J = 14.0 Hz, 2.0 Hz, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.72, 165.61, 165.39, 165.13, 165.07, 164.99, 133.53, 133.49, 133.34, 133.23, 133.09, 133.02, 129.82, 129.76, 129.72, 129.68, 129.51, 129.03, 128.92, 128.91, 128.71, 128.61, 128.50, 128.44, 128.37, 128.27, 128.23, 128.21, 100.55, 94.35, 84.38, 76.89, 76.00, 75.08, 73.85, 73.05, 72.58, 72.04, 70.84, 69.22, 67.78, 66.92, 31.88, 17.84, -1.36. Anal. Calc. For $C_{62}H_{59}Cl_3O_{18}SSi$: C, 59.16; H, 4.72; Cl, 8.45; O, 22.88; S, 2.55; Si, 2.23; Found: C, 59.43; H, 5.06; S, 2.26.

*S-(2,3,4-tri-O-benzoyl-6-O-trichloroethoxycarbonyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-1-S-acetyl-1,6-dithiol-β-D-glucopyranose (**78**)*

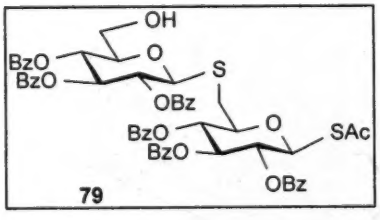


78

Compound **74** (5.24 g, 4.16 mmol) was reacted as described for the conversion of **21** to **30** to give **78** (3.24 g, 64%). Column chromatography (Toluene/ethyl acetate: 15:1). $[\alpha]_D$ – 28.4° (c 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.99-7.20 (m, 30H, aromatic H), 5.88 (dt, 1H, J = 9.5 Hz, 1.5 Hz, H-3), 5.80 (t, 1H, J = 9.5 Hz, H-3'), 5.64-5.58 (m, 2H, H-1, H-2), 5.48 (t, 1H, J = 9.5 Hz,

H-4'), 5.46 (t, 1H, J = 9.5 Hz, H-4), 5.44 (dd, 1H, J = 9.5, 7.0 Hz, H-2'), 5.16 (d, 1H, J = 10.0 Hz, H-1'), 4.86 (d, 1H, J = 12 Hz, OCOOC$\underline{H}_2Cl_3$), 4.82 (d, 1H, J = 12 Hz, OCOOC$\underline{H}_2Cl_3$), 4.47 (dd, 1H, J = 12.0, 6.0 Hz, H-6a'), 4.43 (dd, 1H, J = 12.0, 3.0 Hz, H-6b'), 4.24 (dt, 1H, J = 8.0, 1.0 Hz, H-5), 3.98 (m, 1H, H-5') 3.12 (dd, 1H, J = 15.0, 8.0 Hz, H-6a), 2.79 (dd, 1H, J = 15.0, 2.0 Hz, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.94, 164.03, 163.82, 163.53, 163.48, 163.44, 163.33, 152.09, 131.79, 131.77, 131.73, 131.57, 131.48, 131.46, 128.28, 128.18, 128.12, 128.05, 127.96, 127.35, 127.05, 127.00, 126.96, 126.73, 126.68, 126.64, 126.64, 126.54. 92.66, 81.20, 79.30, 78.70, 74.31, 72.28, 72.24, 70.08, 69.05, 68.18, 67.85, 65.34.29.25, 28.86. Anal. Calc. For $C_{59}H_{49}Cl_3O_{18}S_2$: C, 58.25; H, 4.06; Cl, 8.74; O, 23.67; S, 5.27; Found: C, 58.35; H, 4.36; S, 5.21.

S-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-1-S-acetyl-1,6-dithiol-β-D-glucopyranose ***(79)***

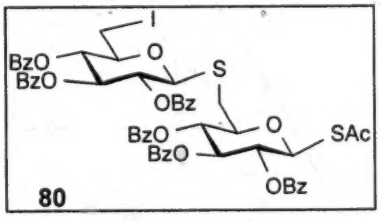


79

To a solution of **78** (3.02 g, 2.48 mmol) in acetic acid (60 mL) was added Zn powder (14 g, 250 mmol), and the mixture was stirred at room temperature for 24 h and then filtered, concentrated. Solution of the residue in CH_2Cl_2 (100 mL) was washed with water, saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel chromatography (Toluene/ethyl acetate: 10:1) to give **79** (2.17 g, 84%) as a clear syrup. $[\alpha]_D$ – 18.9° (c 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.99-7.20 (m, 30H, aromatic H), 5.88 (t, 1H, J = 9.5 Hz, H-3), 5.82 (t, 1H, J = 9.5 Hz, H-3'), 5.62 (t, 1H, J = 9.5 Hz, H-4), 5.62-5.56

(m, 2H, H-1, H-2), 5.44 (t, 1H, J = 9.5 Hz, H-2'), 5.38 (t, 1H, J = 9.5 Hz, H-4'), 5.02 (d, 1H, J = 10.0 Hz, H-1'), 4.26 – 4.22 (m, 1H, H-5), 3.82– 3.74 (m, 2H, H-5', H-6a'), 3.58 (dd, 1H, J = 13.0 Hz, 5.0 Hz, H-6b'), 3.06 (dd, 1H, J = 14.0 Hz, 6.0 Hz, H-6a), 3.00 (dd, 1H, J = 14.0 Hz, 2.0 Hz, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.45, 165.74, 165.64, 165.64, 165.58, 165.51, 165.18, 165.04, 133.66, 133.52, 133.45, 133.26, 133.20, 133,15, 129.97, 129.90, 128.87, 128.74, 128.71, 128.61, 128.53, 128.43, 128.39, 128.34, 128.25, 82.79, 80.45, 79.65, 79.07, 74.13, 74.10, 71.25, 70.77, 69.76, 69.45, 61.60, 30.91, 30.17. Anal. Calcd. For $C_{56}H_{48}Cl_3O_{16}S_2$: C, 64.60; H, 4.65; O, 24.59; S, 6.16; Found: C, 64.47; H, 4.64; S, 6.10.

S-(2,3,4-tri-O-benzoyl-6-O-deoxy-6-iodo-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-1-S-acetyl-1,6-dithiol-β-D-glucopyranose ***(80)***

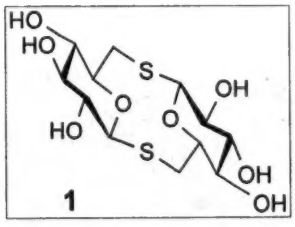


80

A mixture of disaccharide **79** (2.00 g, 1.92 mmol), triphenylphosphine (5.03 g, 19.2 mmol), and carbon tetraiodide (4.99 g, 9.6 mmol), in dry toluene (50 mL), was vigorously stirred at 60 °C for 10 min. The reaction mixture was filtered through a celite pad then concentrated in *vacuo*. The residue was purified by silica gel chromatography (toluene/ethyl acetate: 10:1), which gave **80** (1.77 g, 80%) as a white solid. $[\alpha]_D$ – 36.4 ° (c 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.99-7.20 (m, 30H, aromatic H), 5.92 (t, 1H, J = 9.0 Hz, H-3), 5.76 (t, 1H, J = 9.5 Hz, H-3'), 5.62-5.56 (m, 2H, H-1, H-2), 5.46 (t, 1H, J = 9.5 Hz, H-4), 5.42 (t, 1H, J = 9.5 Hz, H-2'), 5.32 (t, 1H, J = 9.5 Hz, H-4'), 5.18 (d, 1H, J = 10.0 Hz, H-1'), 4.32 (t, 1H, J = 8.0 Hz,

H-5), 3.75 (t, 1H, J = 10.0 Hz, H-5'), 3.34 (d, 1H, J = 10.0 Hz, H-6a'), 3.28 (d, 1H, J = 10.0 Hz, H-6b'), 3.24 (dd, 1H, J = 14.5, 8.0 Hz, H-6a), 2.90 (d, 1H, J = 14.5 Hz, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.17, 165.17, 165.59, 165.32, 165.27, 165.18, 165.08, 133.61, 133.57, 133.23, 133.21, 130,00, 129.93, 129.91, 129.81, 129.68, 128.76, 128.72, 128.65, 128.50, 128.42, 128.36, 128.32, 128.27, 83.04, 80.83, 80.48, 78.00, 73.98, 73.59, 72.74, 71.99, 71.27, 70.01, 31.12, 30.95, 3.12. Anal. Calc. For $C_{56}H_{47}IO_{15}S_2$: C, 58.44; H, 4.12; I, 11.03; O, 20.85; S, 5.57; Found: C, 58.37; H, 4.08; S, 5.55.

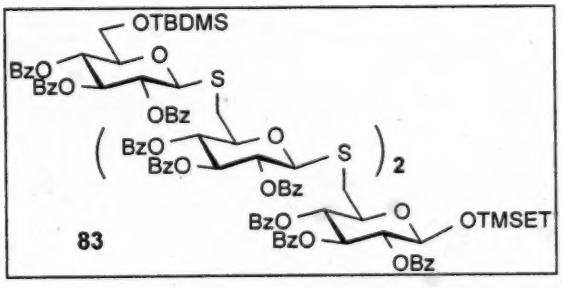
*Cyclo [S-(6-thiol-β-D-glucopyranosyl)-(1→6)]$_2$ (**1**):*



To a solution of disaccharide **80** (161 mg, 0.14 mmol) in dry DMF (2 mL) at –10 °C was added diethylamine (100 μL, 0. 96 mmol) and the solution was then allowed to reach room temperature. After the reaction was complete (TLC, toluene/EtOAc: 6:1) and mixture was concentrated in *vacuo*. The crude residue was subjected to column chromatography (toluene/EtOAc: 20: 1) to give **81** (124 mg, 90%) as a white foam. To a solution of compound **81** (50 mg, 0.051 mmol) in THF (2 mL) and MeOH (30 mL) was added a freshly prepared methanolic solution of NaOMe (1 mM, 1 mL) and stirring was continued at room temperature overnight. The reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated. Solution of the residue in H_2O (20 mL) was washed with ether (3 × 30 mL) and concentrated by co-evaporation with toluene. The crude residue was re-dissolved in H_2O (2 mL), loaded on two Waters C18 Sep-Pak cartridges and then eluted with 10 mL H_2O. The eluant

containing the product was lyophilized, affording **1** as a white solid (18.2 mg, quant.). $[\alpha]_D$ + 2.7° (c 1.2, H_2O); ^1H NMR (400 MHz, DMSO-d6, 100 °C) δ = 4.14 (second order, 1H, $J_{1,2}$ = 9.0 Hz, H-1), 3.42 (m, 1H, H-4), 3.30 (m, 1H, H-5), 3.24-3.18 (m, 2H, H-2, H-3), 2.85 (dd, 1H, J = 15.0, 6.0 Hz, H-6), 2.72 (dd, 1H, J = 15.0, 1.5 Hz, H-6'); ^{13}C NMR (100 MHz, DMSO-d_6, 100 °C) δ 85.34 (C-1), 79.82 (C-5), 77.79 (C-3), 70.83 (C-2), 70.82 (C-4), 32.71 (C-6). HRMS (ESI): Calc. for $C_{12}H_{20}O_8S_2Na$ [M+Na^+] 379.0497, observed 379.0492.

2-(Trimethylsilyl)ethyl S-(2,3,4-tri-O-benzoyl-6-O-tert-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-S-(2, 3, 4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl) (1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)- (1→6)-2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranoside (83)

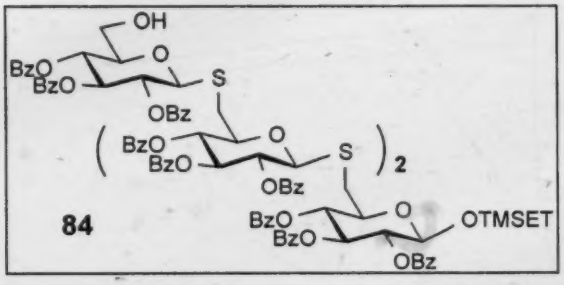


83

To a solution of compound **36** (1.05 g, 0.77 mmol) in MeOH (30 mL) was added a freshly prepared methanolic solution of NaOMe (1 mM, 1 mL). After stirring for 5 h, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated, affording tetrasaccharide **82** (628 mg, quant.). Crude residue **82** was dissolved in pyridine (10 mL). *t*-Butyldimethylsilyl chloride (269 mg, 1.78 mmol) was added slowly to the stirred solution at 0 °C. Stirring continued for 4h during which time the temperature was allowed to reach to rt. After compound **82** had been completely

consumed (TLC, dichloromethane/methanol/H_2O: 65: 35: 2), benzoyl chloride (1.39 mL, 12.0 mmol) was added to the reaction mixture. The reaction was stirred for 24 h then diluted in CH_2Cl_2 (100 mL). The CH_2Cl_2 solution washed with dilute HCl, water, brine, dried (anhydrous Na_2SO_4), filtered, concentrated and followed by column chromatography (toluene/ethyl acetate: 20:1), which yielded **83** as a white solid (1.15 g, 74%). $[\alpha]_D$ + 11.8° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.10-7.20 (m, 60H, aromatic H), 5.97 (t, 1H, J = 10.0 Hz), 5.88 (t, 1H, J = 10.0 Hz), 5.83 (t, 1H, J = 10.0 Hz), 5.78 (t, 1H, J = 10.0 Hz), 5.53 (t, 1H, J = 10.0 Hz, H-4'''), 5.48-5.39 (m, 5H), 5.35 (t, 1H, J = 10.0 Hz), 5.33 (t, 1H, J = 10.0 Hz), 4.98 (d, 1H, J = 10.0 Hz), 4.93 (d, 1H, J = 8.0 Hz, H-1), 4.68 (d, 1H, J = 10.0 Hz), 4.54 (d, 1H, J = 10.0 Hz), 4.24-4.16 (m, 2H), 4.14 (dt, 1H, J = 10.0, 2.5 Hz), 4.06 (dt, 1H, J = 9.5, 3.0 Hz), 4.00 (dt, 1H, J = 10.0, 6.5 Hz, $OC\underline{H}_2CH_2SiMe_3$), 3.92 (dt, 1H, J = 10.0, 3.5 Hz), 3.77 (m, 1H, H-5'''), 3.74-3.69 (m, 2H, H-6a''', H-6b'''), 3.55 (dt, 1H, J = 6.5, 10.0 Hz, $OC\underline{H}_2CH_2SiMe_3$), 3.07 (dd, 1H, J = 14.0, 2.5 Hz), 2.98 (dd, 1H, J = 14.0, 3.0 Hz), 2.96-2.88 (m, 3H), 2.83 (dd, 1H, J = 14.0, 10.0 Hz), 0.96-0.89 (m, 2H, $OCH_2C\underline{H}_2SiMe_3$), 0.84 (s, 9H), -0.3 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.81, 165.73, 165.66, 165.64, 165.61, 165.48, 165.24, 165.21, 165.16, 165.10, 164.97, 164.93, 133.69-132.80, 130.17-128.98, 128.55-128.09 (aromatic), 100.32, 84.88, 84.49, 84.03, 79.56, 78.47, 77.96, 74.42, 74.41, 74.10, 73.94, 73.01, 72.94, 72.59, 72.38, 72.18, 71.11, 70.98, 69.48, 67.62, 63.01, 33.18, 32.04, 25.89, 18.31, 17.82, -1.42, -5.25. ESI-MS Calc. for $C_{119}H_{116}O_{30}S_3Si_2Na$ [M+Na^+] 2199.6, observed 2220.7.

*2-(Trimethylsilyl)ethyl S-(2,3,4-tri-O-benzoyl -β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O- benzoyl-6-thio-β-D-glucopyranoside (**84**)*

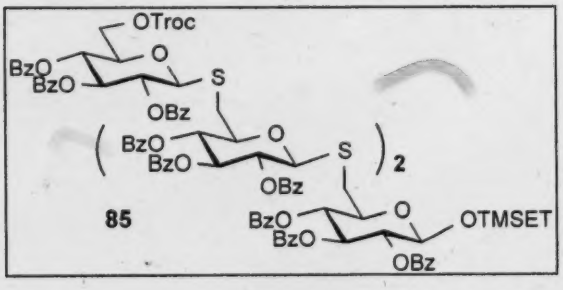


84

The compound **83** (1.0 g, 0.46 mmol) was stirred in 80% aqueous acetic acid (45 mL) at 80 °C for 4 h. The solution was then co-evaporated with toluene to remove water and acetic acid, concentrated and subjected to column chromatography (hexane/ethyl acetate: 2: 3) to give **84** (749 mg, 79%) as a clear syrup. $[\alpha]_D$ + 0.88° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.10-7.20 (m, 60H, aromatic H), 5.98 (t, 1H, J = 10.0 Hz, H-3), 5.91 (t, 1H, J = 10.0 Hz), 5.85 (t, 1H, J = 10.0 Hz, H-3'''), 5.79 (t, 1H, J = 10.0 Hz), 5.66 (t, 1H, J = 10.0 Hz), 5.53 (t, 1H, J = 10.0 Hz), 5.51 (dd, 1H, J = 10.0 Hz, 9.0 Hz, H-2), 5.46 (t, 1H, J = 10.0 Hz), 5.38 (t, 1H, J = 10.0 Hz), 5.33 (t, 1H, J = 10.0 Hz, H-4'''), 5.22 (t, 1H, J = 10.0 Hz), 5.02 (d, 1H, J = 10.0 Hz), 4.99 (d, 1H, J = 8.0 Hz, H-1), 4.71 (d, 1H, J = 10.0 Hz, H-1'''), 4.61 (d, 1H, J = 10.0 Hz), 4.22 (dt, 1H, J = 10.0, 2.0 Hz), 4.16 (dt, 1H, J = 10.0, 3.0 Hz), 4.00 (dt, 1H, J = 10.0, 6.5 Hz, $OC\underline{H}_2CH_2SiMe_3$), 3.95 (dt, 1H, J = 10.0, 3.0 Hz), 3.75-3.69 (m, 2H, H-5''', H-6a'''), 3.62 (dt, 1H, J = 6.5, 10.0 Hz, $OC\underline{H}_2CH_2SiMe_3$), 3.37 (dd, 1H, J = 14.0, 5.0 Hz), 3.20 (dd, 1H, J = 14.0, 3.0 Hz), 3.13 (dd, 1H, J = 14.0, 2.5 Hz), 3.05-2.97 (m, 2H), 2.93 (dd, 1H, J = 14.0, 10.0 Hz), 2.81 (dd, 1H, J = 14.0, 9.5 Hz), 0.96-0.80 (m, 2H, $OCH_2C\underline{H}_2SiMe_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.81, 165.80, 165.74, 165.73, 165.47, 165.42, 165.25, 165.20, 165.16, 165.02,

164.99, 133.67-132.77, 130.17-128.08 (aromatic), 100.31, 84.95, 84.49, 83.01, 79.23, 77.86, 74.25, 74.12, 73.98, 72.97, 72.44, 72.17, 71.67, 71.22, 71.09, 70.55, 69.34, 67.61, 61.29, 33.24, 30.34, 29.68, 17.82, -1.411. ESI-MS Calc. for $C_{113}H_{102}O_{30}S_3SiNa$ [M+Na^+] 2085.5, observed. 2086.5.

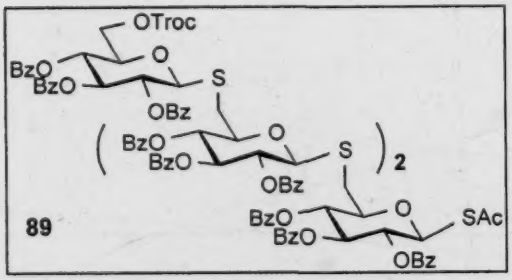
*2-(Trimethylsilyl)ethyl S-(2,3,4-tri-O-benzoyl-6-O-trichloroethoxycarbonyl-β-D-glucopyranosyl)-(1→6)-S-(2, 3, 4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranoside (**85**)*



To a solution of **84** (710 mg, 0.34 mmol) and pyridine (85 μL, 1.02 mmol) in CH_2Cl_2 (30 mL) were added 2, 2, 2-trichloroethoxycarbonyl chloride (TrocCl) (140 μL, 1.02 mmol) and DMAP (10 mg, 0.07 mmol) at 0 °C. After the solution was stirred for 1 h, excess TrocCl was destroyed with MeOH (1 mL). The mixture was concentrated and the residue was dissolved in CH_2Cl_2 (60 mL). The organic solution was washed with 1N HCl, saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel chromatography (hexane/ethyl acetate: 2: 3), which gave **85** (693 mg, 90%) as a crystalline solid. $[\alpha]_D$ + 18.5° (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10-7.20 (m, 60H, aromatic H), 5.98 (t, 1H, J = 10.0 Hz), 5.93 (t, 1H, J = 10.0 Hz), 5.90 (t, 1H, J = 10.0 Hz, H-3'''), 5.77 (t, 1H, J = 10.0 Hz, H-4'''), 5.60 (t, 1H, J = 10.0 Hz), 5.50-5.44 (m, 6H), 5.44 (t, 1H, J = 10.0 Hz), 5.40 (t, 1H, J = 10.0 Hz), 5.09 (d, 1H, J = 10.0

Hz), 4.96 (d, 1H, J = 8.0 Hz, H-1), 4.82 (d, 1H, J = 10.0 Hz), 4.78 (s, 2H, OCOOC$\underline{H}_2Cl_3$), 4.56(d, 1H, J = 10.0 Hz), 4.56 (2s, 2H, H-6a''', H-6b'''), 4.17 (dt, 1H, J = 10.0, 3.0 Hz), 4.13 (dt, 1H, J = 10.0, 3.0 Hz), 4.08-4.04 (m, 2H, H-5'''), 4.00 (dt, 1H, J = 10.0, 6.5 Hz, OC$\underline{H}_2CH_2SiMe_3$), 3.99 (m, 1H), 3.97 (dd, 1H, J = 9.5, 2.5 Hz), 3.57 (dt, 1H, J = 6.5, 10.0 Hz, OC$\underline{H}_2CH_2SiMe_3$), 3.13 (dd, 1H, J = 14.0, 2.5 Hz), 3.06 (dd, 1H, J = 14.0, 2.0 Hz), 2.97 (dd, 1H, J = 10.0, 7.0 Hz), 2.95 (dd, 1H, J = 9.0, 4.0 Hz), 2.89 (dd, 1H, J = 14.0, 9.0 Hz), 2.85 (dd, 1H, J = 14.0, 2.5 Hz), 0.96-0.80 (m, 2H, OCH$_2$C$\underline{H}_2$SiMe$_3$) ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.74, 165.70, 165.65, 165.60, 165.57, 165.23, 165.19, 165.14, 165.02, 164.98, 153.78, 133.39-132.80, 130.04-128.14 (aromatic), 100.24, 94.41, 86.13, 85.66, 85.21, 84.76, 78.24, 77.76, 76.55, 76.11, 74.09, 74.04, 73.96, 73.81, 73.12, 72.98, 72.88, 72.77, 72.52, 72.29, 71.26, 71.23, 71.12, 70.73, 69.38, 67.62, 67.38, 34.61, 34.44, 33.50, 33.05, 21.43, 17.87, -1.42. ESI-MS Calc. for $C_{116}H_{103}Cl_3O_{32}S_3SiNa$ [M+Na$^+$] 2259.4, observed. 2261.5.

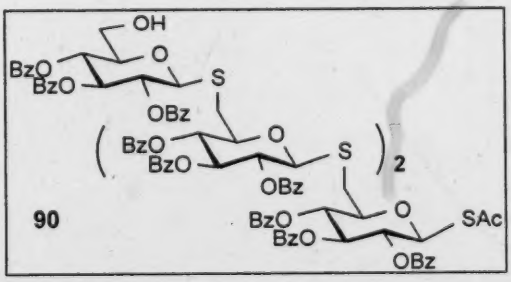
S-(2,3,4-tri-O-benzoyl-6-O-trichloroethoxycarbonyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O- benzoyl-1-S-acetyl-1,6-dithiol-β-D-glucopyranose ***(89)***



Compound **85** (660 mg, 0.29 mmol) was reacted as described for the conversion of **21** to **30** to give **89** (311 mg, 48%). Column chromatography (toluene/ethyl acetate: 10:1). $[\alpha]_D$ + 22.8° (c 1.0, $CHCl_3$); ^{1}H NMR

(500 MHz, $CDCl_3$) δ 8.00-7.10 (m, 60H, aromatic H), 5.95 (t, 1H, J = 10.0 Hz), 5.94 (t, 1H, J = 10.0 Hz), 5.85 (t, 1H, J = 10.0 Hz), 5.84 (t, 1H, J = 10.0 Hz), 5.65 (t, 1H, J = 10.0 Hz, H-1), 5.62 (t, 1H, J = 10.0 Hz, H-2), 5.55 (t, 1H, J = 10.0 Hz), 5.52 (t, 1H, J = 10.0 Hz), 5.51 (t, 1H, J = 10.0 Hz), 5.50 (t, 1H, J = 10.0 Hz), 5.46 (t, 1H, J = 10.0 Hz), 5.14 (d, 1H, J =10.0 Hz), 4.88 (d, 1H, J = 10.0 Hz), 4.82 (d, 1H, J = 12.0 Hz, $OCOOCH_2Cl_3$), 4.81 (d, 1H, J = 10.0 Hz), 4.79 (d, 1H, J = 12.0 Hz, $OCOOCH_2Cl_3$), 4.42 (dd, 1H, J = 12.0, 2.5 Hz, H-6a'''), 4.38 (dd, 1H, J = 12.0, 6.0 Hz, H-6b'''), 4.22 (dd, 1H, J = 10.0, 4.0 Hz), 4.11 (m, 1H, H-5'''), 4.04 (dd, 1H, J = 10.0, 3.0 Hz), 4.00 (dd, 1H, J = 10.0, 4.0 Hz), 3.16 (dd, 1H, J = 14.0, 3.0 Hz), 3.04 (dd, 1H, J = 10.0, 8.0 Hz), 3.04-2.97 (m, 5H), 2.95 (dd, 1H, J = 14.0, 3.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.22, 165.79, 165.64, 165.64, 165.61, 165.43, 165.33, 165.22, 165.19, 165.19, 165.10, 165.07, 153.80, 153.12, 133.79-132.99, 130.05-128.16 (aromatic), 94.42, 93.93, 84.33, 84.29, 83.48, 80.35, 80.12, 78.65, 78.07, 77.33, 75.98, 74.45, 74.18, 74.14, 74.03, 73.88, 72.47, 70.08, 69.35, 67.20, 33.68, 32.11, 31.48, 21.43. ESI-MS Calc. for $C_{113}H_{93}Cl_3O_{32}S_4Na$ [M+Na^+] 2217.3, observed. 2216.9.

S-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-1-S-acetyl-1,6-dithiol-β-D-glucopyranose ***(90)***



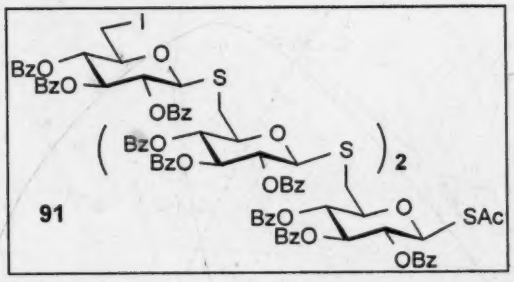
To a solution of **89** (280 mg, 0.13 mmol) in acetic acid (50 mL) was added Zn powder (728 mg, 13 mmol), and the mixture was stirred at room temperature for 24 h and then

filtered, concentrated. A solution of the residue in CH_2Cl_2 (100 mL) was washed with water, saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel chromatography (toluene/ethyl acetate: 10:1) to give **90** (198 mg, 77%) as a syrup. $[\alpha]_D$ + 12.6° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.00-7.10 (m, 60H, aromatic H), 5.96 (t, 1H, J = 10.0 Hz, H-3), 5.88 (t, 1H, J = 10.0 Hz), 5.85 (t, 1H, J = 10.0 Hz), 5.83 (t, 1H, J = 10.0 Hz), 5.68 (t, 1H, J = 10.0 Hz, H-1), 5.66 (t, 1H, J = 10.0 Hz), 5.63 (dd, 1H, J = 10.0, 9.9 Hz, H-2), 5.57 (t, 1H, J = 10.0 Hz), 5.50 (t, 1H, J = 10.0 Hz, H-2'''), 5.48 (d, 1H, J = 10.0 Hz, H-1), 5.45 (t, 1H, J = 10.0 Hz), 5.39 (t, 1H, J = 10.0 Hz), 5.30 (t, 1H, J = 10.0 Hz, H-4'''), 5.08 (d, 1H, J = 10.0 Hz, H-1'''), 4.80 (d, 1H, J = 10.0 Hz), 4.76 (d, 1H, J = 10.0 Hz), 4.24 (dt, 1H, J = 10.0, 3.0 Hz, H-5), 4.08-4.01 (m, 2H), 4.02 (dd, 1H, J = 10.0, 3.0 Hz), 3.77 (m, 1H, H-5), 3.73 (dd, 1H, J = 13.0, 2.0 Hz, H-6a'''), 3.45 (dd, 1H, J = 13.0, 5.0 Hz, H-6b'''), 3.17 (dd, 1H, J = 13.0, 3.0 Hz), 3.08 (dd, 1H, J = 14.0, 3.0 Hz), 3.04 (dd, 1H, J = 10.0, 2.0 Hz), 3.02 (m, 1H), 2.98 (dd, 1H, J = 14.0, 8.5 Hz), 2.95 (dd, 1H, J = 14.0, 8.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.20, 165.85, 165.72, 165.65, 165.54, 165.49, 165.36, 165.25, 165.25, 165.15, 165.10, 165.09, 133.47-132.06, 130.03-128.22, 125.29, 84.18, 83.69, 83.06, 80.41, 79.87, 78.19, 77.91, 74.25, 74.17, 74.08, 74.00, 72.37, 72.02, 71.66, 71.20, 71.09, 70.69, 70.18, 69.40, 61.44, 33.68, 32.39, 31.72, 30.85, 30.51. ESI-MS Calc. for $C_{113}H_{93}Cl_3O_{32}S_4Na$ $[M+Na^+]$ 2217.3, observed. 2216.9.

S-(2,3,4-tri-O-benzoyl-6-O-deoxy-6-iodo -β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-

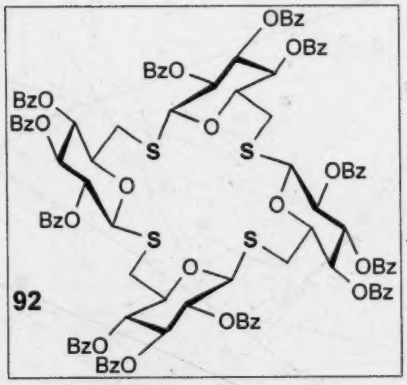
glucopyranosyl -(1→6)-2,3,4-tri-O benzoyl-1-S-acetyl-1,6-dithiol-β-D-glucopyranose

(91)



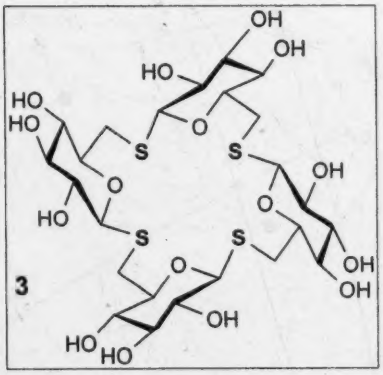
A mixture of tetrasaccharide **90** (160 mg, 0.079 mmol), triphenylphosphine (207 mg, 0.79 mmol), and carbon tetraiodide (205.4 mg, 0.395 mmol), in dry toluene (10 mL), was vigorously stirred at 60 °C for 10 min. The reaction mixture was filtered through a celite pad then concentrated in *vacuo*. The residue was purified by silica gel chromatography (toluene/ethyl acetate: 10:1), which gave **91** (137 mg, 81%) as a crystalline solid. $[\alpha]_D$ + 11.1° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.00-7.10 (m, 60H, aromatic H), 5.97 (t, 1H, J = 10.0 Hz, H-3), 5.91 (t, 1H, J = 10.0 Hz), 5.90 (t, 1H, J = 10.0 Hz), 5.87 (t, 1H, J = 10.0 Hz), 5.68 (d, 1H, J = 10.0 Hz, H-1), 5.62 (dd, 1H, J = 10.0, 9.5 Hz, H-2), 5.56-5.44 (m, 7H) 5.18 (d, 1H, J = 10.0 Hz), 4.90 (d, 1H, J = 10.0 Hz), 4.81 (d, 1H, J = 10.0 Hz), 4.25 (m, 1H), 4.20 (dt, 1H, J = 10.0, 3.0 Hz), 4.08 (dt, 1H, J = 10.0, 3.0 Hz), 3.82 (dt, 1H, J = 10.0, 2.0 Hz, H-5'''), 3.32 (dd, 1H, J = 14.0, 2.0 Hz, H-6a'''), 3.19 (dd, 1H, J = 14.0, 10.0 Hz, H-6b'''), 3.11 (dd, 1H, J = 10.0 Hz, 9.5 Hz), 3.09 (d, 1H, J = 3.0 Hz), 3.05 (2s, 2H), 3.01 (dd, 1H, J = 14.0, 9.5 Hz), 2.97 (dd, 1H, J = 14.0, 2.5 Hz). ^{13}C NMR (125 M Hz, $CDCl_3$) δ 192.10, 165.77, 165.68, 165.63, 165.48, 165.44, 165.33, 165.27, 165.23, 165.11, 165.05, 165.01, 133.47-132.96, 130.00-128.14, 84.81, 83.63, 80.36, 79.90, 78.79, 78.21, 78.02, 74.14, 74.00, 72.36, 72.61, 72.53, 72.50, 72.10, 71.47, 71.20, 71.05, 70.18, 33.31, 33.11, 31.77, 30.85, 3.74. ESI-MS Calc. for $C_{110}H_{91}IO_{29}S_4Na$ $[M+Na^+]$ 2153.3, observed. 2154.3.

Cyclo [S-(2,3,4-tri-O-benzoyl-6-thiol-β-D-glucopyranosyl)-(1→6)]$_4$ ***(92)***:



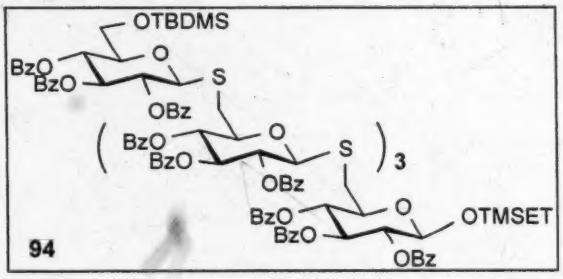
To a solution of linear tetrasaccharide **91** (114 mg, 0.054mmol) in dry DMF (5 mL) at –20 °C was added diethylamine (40 μL, 0.38 mmol). The solution was stirred for 30 min at 0 °C then allowed to reach room temperature. After 3 h, the reaction was complete (TLC, toluene:/ethyl acetate: 1:1) and mixture was concentrated in *vacuo*. Solution of the residue in ether (50 mL), was washed with H_2O (3 × 50 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and subjected to column chromatography (toluene/EtOAc: 20:1) to give **92** (95 mg, 95%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.90-7.20 (m, 15Hz, aromatic), 5.92 (d, 1H, J = 10.0 Hz, H-3), 5.59 (t, 1H, J = 9.5 Hz, H-2), 5.55 (t, 1H, J = 9.5 Hz, H-4), 5.20 (b, 1H, H-1), 4.50 (b, 1H, H-5), 3.40 (dd, 1H, J = 14.0 Hz, J = 7.0 Hz, H-6a), 3.20 (d, 1H, J = 14.0 Hz, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.80, 164.99, 164.96, 133.11, 133.04, 132.95, 129.84, 129.76, 129.73, 129.34, 129.00, 128.87, 128.24, 128.20, 128.18, 77.27, 77.02, 76.76, 74.01, 71.98, 71.22, 31.35. ESI-MS Calc. for $C_{108}H_{88}O_{28}S_4Na$ [M+Na$^+$] 1983.4, observed. 1983.9.

Cyclo [S-(6-thiol-β-D-glucopyranosyl)-(1→6)]$_4$ (3):



To a solution of compound **92** (95 mg, 0.051 mmol) in and THF (1 mL) and MeOH (10 mL) was added a freshly prepared methanolic solution of NaOMe (1 mM, 0.5 mL) and stirring was continued at room temperature overnight. The reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated. Solution of the residue in H_2O (20 mL) was washed with ether (3 × 20 mL) and concentrated by co-evaporation with toluene. The crude residue was re-dissolved in H_2O (4 mL), loaded on four Waters C18 Sep-Pak cartridges and then eluted with 20 mL H_2O. The eluant containing the product was lyophilized, affording **3** as a white solid (35 mg, 95%). $[\alpha]_D$ + 10.0° (c 0.7, H_2O); ^{1}H NMR (600 MHz, D_2O, 27 °C) δ = 4.70 (d, 1H, J = 10.0 Hz, H-1), 3.75 (dt, 1H, J = 9.0, 1.5 Hz, H-5), 3.49 (t, 1H, J = 9.0 Hz, H-3), 3.32 (t, 1H, J = 9.5 Hz, H-2), 3.31 (t, 1H, J = 9.5 Hz, H-4), 3.24 (d, 1H, J = 14.0 , 2.0 Hz, H-6), 2.98 (dd, 1H, J = 14.0, 8.5 Hz, H-6'); ^{13}C NMR (125 MHz, D_2O, 27 °C) δ 86.19, 80.22, 77.78, 73.33, 73.26, 31.60. HRMS (ESI): Calc. for $C_{24}H_{40}O_{16}S_4Na$ [M+Na^+] 735.1097, observed 735.1093.

*2-(Trimethylsilyl)ethyl S-(2,3,4-tri-O-benzoyl-6-O-tert-butyldimethylsilyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O- benzoyl-6-thio-β-D-glucopyranoside (**94**)*

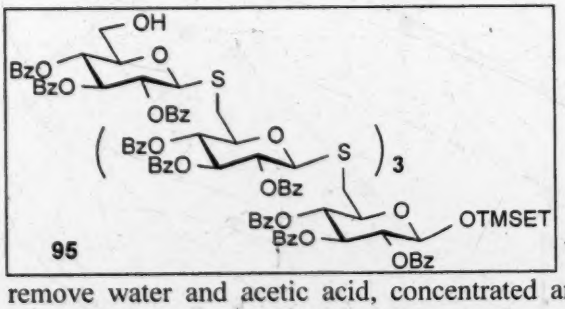


94

To a solution of compound **41** (1.73g, 1.04 mmol) in MeOH (100 mL) was added a freshly prepared methanolic solution of NaOMe (1 mM, 5 mL). After stirring for 5 h, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated, affording pentasaccharide **93** (1.04 g, quant.). Crude residue **93** was dissolved in pyridine (20 mL). *t*-Butyldimethylsilyl chloride (393 mg, 2.6 mmol) was added slowly to the stirred solution at 0 °C. Stirring continued for 4h during which time the temperature was allowed to reach to rt. After compound **93** had been completely consumed (TLC, dichloromethane/methanol/H_2O: 65:35:5), benzoyl chloride (2.17 mL, 18.72 mmol) was added to the reaction mixture. The reaction was stirred for 24 h then diluted in CH_2Cl_2 (50 mL). The CH_2Cl_2 solution washed with dilute HCl, water, brine, dried (anhydrous Na_2SO_4), filtered, concentrated and followed by column chromatography (toluene/ethyl acetate: 10:1), which yielded **94** as a white solid (2.24 g, 81%). $[\alpha]_D$ + 26.9° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.10-7.20 (m, 90H, aromatic H), 5.99 (t, 1H, J = 10.0 Hz), 5.97 (t, 1H, J = 10.0 Hz), 5.95 (t, 1H, J = 10.0 Hz), 5.84 (t, 1H, J = 10.0 Hz), 5.81 (t, 1H, J = 10.0 Hz), 5.58 (t, 1H, J = 10.0 Hz), 5.52 (t, 1H, J = 10.0 Hz), 5.50 (t, 1H, J = 10.0 Hz), 5.48 (t, 1H, J = 10.0 Hz), 5.47 (t, 1H, J = 10.0 Hz), 5.43 (t, 1H, J = 10.0 Hz), 5.42 (t, 1H, J = 10.0 Hz), 5.37 (t, 1H, J = 10.0 Hz), 5.34 (t, 1H, J = 10.0 Hz), 5.32 (t, 1H, J = 10.0 Hz), 5.18 (d, 1H, J = 10.0 Hz), 4.99 (d, 1H, J = 8.0 Hz, H-1), 4.86 (d, 1H, J = 10.0 Hz), 4.63 (d, 1H, J = 10.0 Hz), 4.58 (d, 1H, J = 10.0 Hz), 4.21 (dd, 1H, J = 10.0, 2.0 Hz), 4.18-4.11 (m, 2H), 4.01 (dt, 1H, J = 10.0, 6.5 Hz, $OC\underline{H_2}CH_2SiMe_3$), 3.94 (dt,

1H, J = 10.0, 3.0 Hz), 3.78-3.64 (m, 3H), 3.61 (dt, 1H, J = 10.0, 6.5 Hz, $OC\underline{H_2}CH_2SiMe_3$), 3.13 (dd, 1H, J = 14.0, 2.5 Hz), 3.03-2.88 (m, 3H), 2.85 (dd, 1H, J = 14.0, 2.5 Hz), 2.80 (dd, 1H, J = 14.0, 9.5 Hz), 0.96-0.89 (m, 2H, $OCH_2C\underline{H_2}SiMe_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.85, 165.82, 165.79, 165.78, 165.75, 165.72, 165.52, 165.41, 165.30, 165.25, 165.12, 165.06, 165.01, 164.98, 133.39-132.86, 130.17-128.18 (aromatic), 100.26, 85.66, 85.55, 84.88, 84.72, 84.27, 79.56, 78.22, 78.00, 77.73, 77.24, 76.99, 76.73, 74.36, 74.11, 74.07, 73.95, 73.84, 73.03, 73.02, 72.94, 72.83, 72.80, 72.53, 72.26, 71.37, 71.21, 71.07, 70.85, 69.63, 67.61, 63.15, 34.28, 34.19, 33.35, 32.41, 25.83, 21.43, 18.29, 17.85, -1.42, -5.27, -5.40. ESI-MS Calc. for $C_{146}H_{138}O_{37}S_4Si_2Na$ $[M+Na^+]$ 2689.7, observed. 2691.

*2-(Trimethylsilyl)ethyl S-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O- benzoyl-6-thio-β-D-glucopyranoside (**95**)*

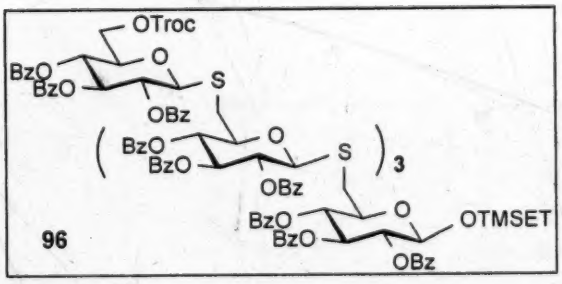


95

The compound **94** (1.83 g, 0.69 mmol) was stirred in 80% aqueous acetic acid (40 mL) at 80 °C for 3.5 h. The solution was then co-evaporated with toluene to remove water and acetic acid, concentrated and subjected to column chromatography (hexane/ethyl acetate: 1: 2) to give **95** (1.21 g, 69%) as a clear syrup. $[\alpha]_D$ + 20.2° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.10-7.20 (m, 90H, aromatic H), 6.06 (t, 1H, J =

10.0 Hz), 6.01 (t, 1H, J = 10.0 Hz), 5.95 (t, 1H, J = 10.0 Hz), 5.91 (t, 1H, J = 10.0 Hz), 5.86 (t, 1H, J = 10.0 Hz), 5.82 (t, 1H, J = 10.0 Hz), 5.60 (t, 1H, J = 10.0 Hz), 5.53 (t, 1H, J = 10.0 Hz), 5.51-5.46 (m, 3H), 5.40 (t, 1H, J = 10.0 Hz), 5.39 (t, 1H, J = 10.0 Hz), 5.35 (t, 1H, J = 10.0 Hz), 5.31 (t, 1H, J = 10.0 Hz), 5.13 (d, 1H, J = 10.0 Hz), 5.12 (t, 1H, J = 10.0 Hz), 4.91 (d, 1H, J = 10.0 Hz), 4.88 (t, 1H, J = 10.0 Hz), 4.71 (d, 1H, J = 8.0 Hz, H-1), 4.69 (d, 1H, J = 10.0 Hz), 4.43 (dt, 1H, J = 10.0, 3.0 Hz), 4.32 (dt, 1H, J = 10.0, 3.0 Hz), 4.23 (dt, 1H, J = 10.0, 3.0 Hz), 4.04 (dt, 1H, J = 10.0, 6.5 Hz, $OC\underline{H}_2CH_2SiMe_3$), 3.88 (dt, 1H, J = 10.0, 3.0 Hz), 3.69-3.63 (m, 3H), 3.24 (dd, 1H, J = 12.0, 2.0 Hz), 3.20 (dd, 1H, J = 14.0, 2.5 Hz), 3.11 (dd, 1H, J = 14.0, 10.0 Hz), 3.00 (dd, 1H, J = 15.0, 10.0 Hz), 2.94 (dd, 1H, J = 15.0, 10.0 Hz), 2.79 (dd, 1H, J = 15.0, 10.0 Hz), 2.76 (dd, 1H, J = 12.0, 2.0 Hz), 2.58 (dd, 1H, J = 10.0, 5.0 Hz), 0.98-0.89 (m, 2H, $OCH_2C\underline{H}_2SiMe_3$). ESI-MS Calc. for $C_{140}H_{124}O_{37}S_4SiNa$ $[M+Na^+]$ 2575.6, observed. 2577.

2-(Trimethylsilyl)ethyl S-(2,3,4-tri-O-benzoyl-6-O-trichloroethoxycarbonyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O- benzoyl-6-thio-β-D-glucopyranoside ***(96)***

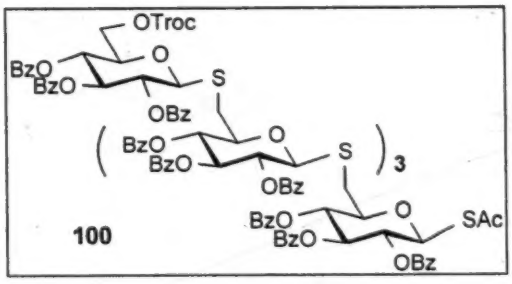


96

To a solution of **95** (1.08 g, 0.42 mmol) and pyridine (102 μL, 1.26 mmol) in CH_2Cl_2 (10 mL) were added 2, 2, 2-trichloroethoxycarbonyl chloride (TrocCl) (174 μL, 1.26 mmol) and

DMAP (14 mg, 0.084 mmol) at 0 °C. After the solution was stirred for 1 h, excess TrocCl was destroyed with MeOH (5 mL). The mixture was concentrated and the residue was dissolved in CH_2Cl_2 (20 mL). The organic solution was washed with 1N HCl, saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel chromatography (hexane/ethyl acetate: 1:2), which gave **96** (1.08 g, 94%) as a crystalline solid. $[\alpha]_D$ + 33.6° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.10-7.20(m, 90H, aromatic H), 6.01 (t, 1H, J = 10.0 Hz), 5.99 (t, 1H, J = 10.0 Hz), 5.97 (t, 1H, J = 10.0 Hz), 5.89 (t, 1H, J = 10.0 Hz), 5.83 (t, 1H, J = 10.0 Hz), 5.62 (t, 1H, J = 10.0 Hz), 5.56 (t, 1H, J = 10.0 Hz), 5.53-5.46 (m, H), 5.43 (t, 1H, J = 10.0 Hz), 5.38 (t, 1H, J = 10.0 Hz), 5.35 (t, 1H, J = 10.0 Hz), 5.34 (t, 1H, J = 10.0 Hz), 5.27 (d, 1H, J = 10.0 Hz), 5.02 (d, 1H, J = 8.0 Hz, H-1), 5.01 (d, 1H, J = 10.0 Hz), 4.78 (d, 2H, J = 3.0 Hz, Troc), 4.74 (d, 1H, J = 10.0 Hz), 4.70 (d, 1H, J = 10.0 Hz), 4.34-4.21(m, 4H), 4.17 (dt, 1H, J = 10.0, 3.0 Hz), 4.06-3.98(m, 3H), 3.63 (dt, 1H, J = 10.0, 6.5 Hz, $OC\underline{H_2}CH_2SiMe_3$), 3.16 (dd, 1H, J = 14.0, 2.5 Hz), 3.07 (dd, 1H, J = 14.0, 2.5 Hz), 3.05 (dd, 1H, J = 14.0, 10.0 Hz), 2.98 (dd, 1H, J = 14.0, 10.0 Hz), 2.96-2.88 (m, 3H), 2.83 (dd, 1H, J = 14.0, 10.0 Hz), 0.96-0.89 (m, 2H, $OCH_2C\underline{H_2}SiMe_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.82, 165.79, 165.74, 165.67, 165.62, 165.60, 165.54, 165.35, 165.18, 165.08, 165.06, 164.92 164.87, 153.73, 133.45-133.95, 129.99-128.12 (aromatic), 100.31, 94.41, 84.82, 84.80, 84.63, 83.01, 78.63, 77.81, 76.08, 74.31, 74.07, 73.96, 73.82, 73.02, 72.98, 70.89, 69.34, 67.63, 67.33, 33.20, 33.15, 32.65, 29.68, 17.82, -1.42. ESI-MS Calc. for $C_{143}H_{125}Cl_3O_{39}S_4SiNa$ $[M+Na^+]$ 2749.5, observed. 2753.0.

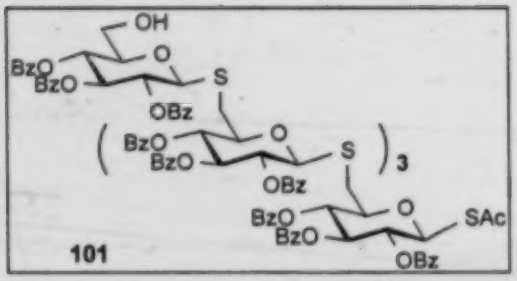
S-(2,3,4-tri-O-benzoyl-6-O-trichloroethoxycarbonyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O- benzoyl-1-S-acetyl-1,6-dithiol-β-D-glucopyranose ***(100)***



100

Compound **96** (1.01 g, 0.37 mmol) was reacted as described for the conversion of **21** to **30** to give **100** (440 mg, 41%). Column chromatography (toluene/ethyl acetate: 10: 1). $[\alpha]_D$ + 29.1° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.10-7.20 (m, 90H, aromatic H), 5.98 (t, 1H, J = 10.0 Hz), 5.94 (t, 1H, J = 10.0 Hz), 5.93 (t, 1H, J = 10.0 Hz), 5.89 (t, 1H, J = 10.0 Hz), 5.83 (t, 1H, J = 10.0 Hz), 5.64 (d, 1H, J = 10.0 Hz, H-1), 5.64-5.40 (m, 9H), 5.20 (d, 1H, J = 10.0 Hz), 5.05 (d, 1H, J = 8.0 Hz, H-1), 4.84 (d, 1H, J = 10.0 Hz), 4.80 (d, 1H, J = 12.0 Hz, $OCOOCH_2Cl_3$), 4.76 (d, 1H, J = 12.0 Hz, $OCOOCH_2Cl_3$), 4.72 (d, 1H, J = 10.0 Hz), 4.38 (dd, 1H, J = 12.0, 2.5 Hz, H-6a''''), 4.32 (dd, 1H, J = 12.0, 5.5 Hz, H-6b''''), 4.23 (dd, 1H, J = 10.0, 2.5 Hz), 4.19 (dd, 1H, J = 10.0, 6.0 Hz), 4.13-4.00 (m, 3H), 3.17-2.90 (m, 8H), 2.31 (s, 3H, $SCO\underline{CH_3}$). ESI-MS Calc. for $C_{140}H_{115}Cl_3O_{39}S_5Na$ $[M+Na^+]$ 2707.5, observed. 2711.

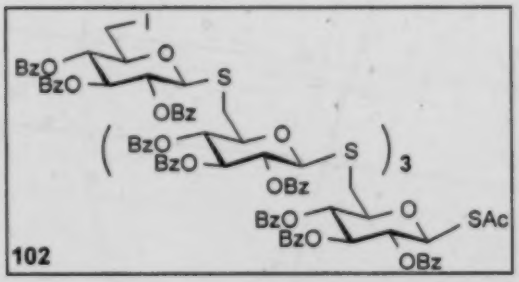
S-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-1-S-acetyl-1,6-dithiol-β-D-glucopyranose ***(101)***



101

To a solution of **100** (370 mg, 0.14 mmol) in acetic acid (20 mL) was added Zn powder (770 mg, 14 mmol), and the mixture was stirred at room temperature for 48 h and then filtered, concentrated. A solution of the residue in CH_2Cl_2 (50 mL) was washed with water, saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel chromatography (toluene/ethyl acetate: 10:1) to give **101** (262 mg, 76%) as a syrup. $[\alpha]_D$ + 22.9° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.10-7.20 (m, 90H, aromatic H), 5.97 (t, 1H, J = 10.0 Hz), 5.96 (t, 1H, J = 10.0 Hz), 5.92 (t, 1H, J = 10.0 Hz), 5.86 (t, 1H, J = 10.0 Hz), 5.85 (t, 1H, J = 10.0 Hz), 5.73 (t, 1H, J = 10.0 Hz), 5.70 (d, 1H, J = 10.0 Hz, H-1), 5.62 (dd, 1H, J =10.0, 9.5 Hz), 5.55-5.48 (m, 3H), 5.47 (t, 1H, J = 10.0 Hz), 5.44 (t, 1H, J = 10.0 Hz), 5.43 (t, 1H, J = 10.0 Hz), 5.28 (t, 1H, J = 10.0 Hz, H-2), 5.26 (d, 1H, J = 10.0 Hz), 5.14 (t, 1H, J = 10.0 Hz, H-4''''), 4.96 (d, 1H, J = 10.0 Hz), 4.72 (d, 1H, J = 10.0 Hz), 4.70 (d, 1H, J = 10.0 Hz), 4.32-4.22 (m, 2H), 4.10 (dt, 1H, J = 10.0, 3.0 Hz), 3.96 (dt, 1H, J = 10.0, 2.5 Hz), 3.76-3.66 (m, 2H, H-5'''', H-6a''''), 3.30 (dd, 1H, J = 12.0 Hz, 5.5 Hz), 3.21 (dd, 1H, J = 7.0, 3.0 Hz), 3.19 (dd, 1H, J = 5.0, 1.5 Hz), 3.06 (d, 1H, J = 8.0 Hz), 3.02 (d, 1H, J = 9.5 Hz), 2.96-2.86 (m, 5H), 2.31 (s, 3H, SCO$\underline{CH_3}$). ESI-MS Calc. for $C_{137}H_{114}O_{37}S_5Na$ [M+Na^+] 2533.6, observed. 2535.

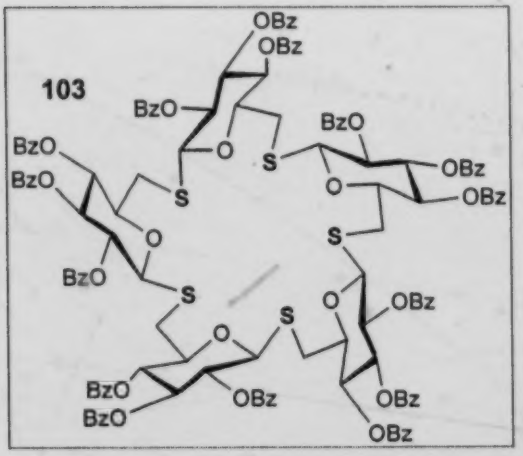
*S-(2,3,4-tri-O-benzoyl--6-O-deoxy-6-iodo-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-S-(2,3,4-tri-O-benzoyl-6-thio-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-1-S-acetyl-1,6-dithiol-β-D-glucopyranose (**102**)*



102

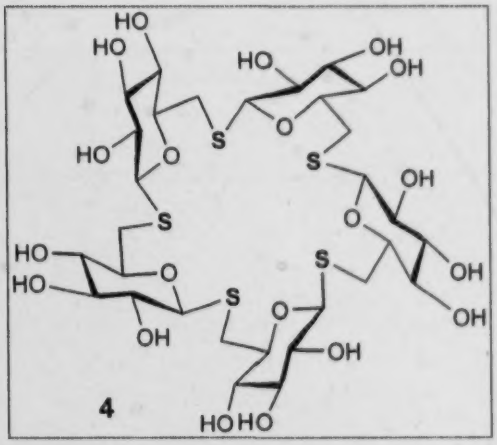
A mixture of trisaccharide **101** (122 mg, 0.049 mmol), triphenylphosphine (127 mg, 0.49 mmol), and carbon tetraiodide (126 mg, 0.24 mmol), in dry toluene (20 mL), was vigorously stirred at 60 °C for 10 min. The reaction mixture was filtered through a celite pad then concentrated in *vacuo*. The residue was purified by silica gel chromatography (toluene/ethyl acetate: 10:1), which gave **102** (97 mg, 77%) as a crystalline solid. $[\alpha]_D$ + 38.8° (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.10-7.20 (m, 90H, aromatic H), 6.05 (t, 1H, J = 10.0 Hz), 5.99 (t, 1H, J = 10.0 Hz), 5.92 (t, 1H, J = 10.0 Hz), 5.91 (t, 1H, J = 10.0 Hz), 5.87 (t, 1H, J = 10.0 Hz), 5.72 (d, 1H, J = 10.0 Hz, H-1), 5.63 (t, 1H, J = 10.0 Hz, H-2), 5.55 (t, 1H, J = 10.0 Hz), 5.54 (t, 1H, J = 10.0 Hz), 5.51 (t, 1H, J = 10.0 Hz), 5.50 (t, 1H, J = 10.0 Hz), 5.49 (t, 1H, J = 10.0 Hz), 5.47 (t, 1H, J = 10.0 Hz), 5.45 (t, 1H, J = 10.0 Hz), 5.44 (t, 1H, J = 10.0 Hz), 5.37 (t, 1H, J = 10.0 Hz), 5.26 (d, 1H, J = 10.0 Hz), 5.12 (d, 1H, J = 10.0 Hz), 4.84 (d, 1H, J = 10.0 Hz), 4.74 (d, 1H, J = 10.0 Hz), 4.34-4.25 (m, 2H), 4.20 (dt, 1H, J = 10.0, 2.5 Hz), 4.10 (m, 1H), 3.80 (dt, 1H, J = 10.0, 2.0 Hz, H-5''''), 3.28 (dd, 1H, J = 12.0, 2.0 Hz, H-6a''''), 3.20-3.10 (m, 2H), 3.02-2.98 (m, 6H), 2.31 (s, 3H, $SCO\underline{CH_3}$). ESI-MS Calc. for $C_{137}H_{113}IO_{36}S_5Na$ $[M+Na^+]$ 2643.5, observed. 2645.

Cyclo [S-(2,3,4-tri-O-benzoyl-6-thiol-β-D-glucopyranosyl)-(1→6)]$_5$ ***(103)***:



To a solution of linear pentasaccharide **102** (71 mg, 0.027mmol) in dry DMF (3 mL) at –20 °C was added diethylamine (20 μL, 0.19 mmol). The solution was stirred for 30 min at 0 °C then allowed to reach room temperature. After 4 h, the reaction was complete (TLC, toluene:/ethyl acetate: 1:1) and mixture was concentrated in *vacuo*. Solution of the residue in ether (30 mL), was washed with H_2O (3 × 30 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and subjected to column chromatography (toluene/EtOAc: 20: 1) to give **103** (56 mg, 84%) as a white foam. $[\alpha]_D$ + 7.1° (c 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.90-7.10 (m, 15 Hz, aromatic), 5.85 (t, 1H, J = 9.5 Hz, H-3), 5.70 (t, 1H, J = 9.5 Hz, H-2), 5.56 (t, 1H, J = 9.5 Hz, H-4), 4.82 (d, 1H, 10.0 Hz, H-1), 4.50 (t, 1H, J = 10.0 Hz, H-5), 3.40 (dd, 1H, J = 13.5, 3.0 Hz, H-6a), 3.20 (dd, 1H, J = 13.5, 3.0 Hz, H-6b). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.74, 165.28, 164.87, 133.05, 132.94, 130.00, 129.92, 129.75, 129.39, 129.14, 128.97, 128.26, 128.10, 82.51, 78.64, 74.16, 72.34, 70.89, 31.45. ESI-MS Calc. for $C_{128}H_{106}O_{34}S_5Na$ [M+Na^+] 2475.6, observed. 2475.

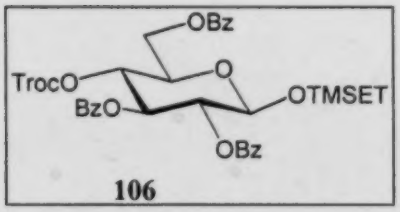
Cyclo [S-(6-thiol-β-D-glucopyranosyl)-(1→6)]$_5$ (4):



4

To a solution of compound **103** (56 mg, 0.023 mmol) in and THF (1 mL) and MeOH (10 mL) was added a freshly prepared methanolic solution of NaOMe (1 mM, 0.5 mL) and stirring was continued at room temperature overnight. The reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated. Solution of the residue in H_2O (20 mL) was washed with ether (3 × 20 mL) and concentrated by co-evaporation with toluene. The crude residue was re-dissolved in H_2O (4 mL), loaded on two Waters C18 Sep-Pak cartridges and then eluted with 20 mL H_2O. The eluant containing the product was lyophilized, affording **4** as a white solid (20 mg, quant.). $[\alpha]_D$ + 25.6° (c 0.8, H_2O); ^{1}H NMR (600 MHz, D_2O, 27 °C) δ = 4.70 (d, 1H, J = 10.0 Hz, H-1), 3.75 (dt, 1H, J = 9.0 Hz, J = 1.5 Hz, H-5), 3.49 (t, 1H, J = 9.0 Hz, H-3), 3.32 (t, 1H, J = 9.5 Hz, H-2), 3.31 (t, 1H, J = 9.5 Hz, H-4), 3.24 (d, 1H, J = 14.0, 2.0 Hz, H-6a), 2.98 (dd, 1H, J = 14.0, 8.5 Hz, H-6b); ^{13}C NMR (125 MHz, D_2O, 27 °C) δ 86.71, 80.31, 77.91, 73.74, 73.48, 32.91. HRMS (ESI): Calc. for $C_{30}H_{50}O_{20}S_5Na$ [M+Na^+] 913.1397, observed 913.1398.

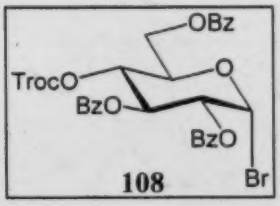
2-(Trimethylsilyl)ethyl 2,3,6-tri-O-benzoyl-4-O-trichloroethoxycarbonyl-β-D-glucopyranoside (106)



106

To a solution of **104** (2.50 g, 4.22 mmol) and pyridine (1.0 mL) in CH_2Cl_2 (100 mL) were added 2, 2, 2-trichloroethoxycarbonyl chloride (TrocCl) (1.8 mL, 12.7 mmol) and DMAP (67 mg, 0.1 mmol) at 0 °C. After the solution was stirred for 1 h, excess TrocCl was destroyed with MeOH (5 mL). The mixture was concentrated and the residue was dissolved in CH_2Cl_2 (300 mL). The organic solution was washed with 1N HCl, saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel chromatography (hexane/ethyl acetate: 2:1), which gave **106** (3.05 g, 94%) as a crystalline solid. $[\alpha]_D$ + 40.8° (c 1.0, $CHCl_3$); ^{1}H NMR *(600 MHz, $CDCl_3$)* δ 5.78 (t, 1H, J = 10.0 Hz, H-3), 5.44 (dd, 1H, J = 10.0, 8.0 Hz, H-2), 5.32 (t, 1H, J = 10.0 Hz, H-4), 4.80 (d, 1H, J = 8.0 Hz, H-1), 4.63 (dd, 1H, J = 12.0, 3.0 Hz, H-6a), 4.62 (d, 1H, J = 12 Hz, OCOOC$\underline{H_2}Cl_3$), 4.54 (dd, 1H, J = 12.0, 5.0 Hz, H-6b), 4.43 (d, 1H, J = 12 Hz, OCOOC$\underline{H_2}Cl_3$), 4.10 – 4.06 (m, 1H, H-5), 3.99 (dt, 1H, J = 10.0, 6.0 Hz, OC$\underline{H_2}CH_2SiMe_3$), 3.57 (dt, 1H, J = 10.0, 6.5Hz, $OCH_2C\underline{H_2}SiMe_3$), 0.96-0.83 (m, 2H, $OCH_2C\underline{H_2}SiMe_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.04, 166.44, 165.03, 153.134, 133.4, 133.27, 133.14, 129.90, 129.80, 129.78, 129.55, 129.32, 128.46, 128,35, 128.28, 100.59, 93.91, 77.32, 76.81, 73.89, 72.80, 71.84, 71.43, 67.78, 62.60, 17.91, -1.52. HRMS (ESI): Calc. for Na $[M+Na^+]$ $C_{35}H_{37}Cl_3O_{11}SiNa$ 789.1063, observed 789.1066.

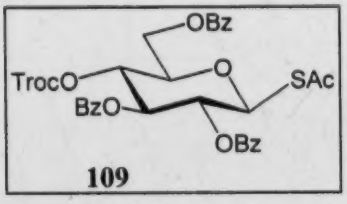
2,3,6-tri-O-benzoyl-4-O-trichloroethoxycarbonyl-β-D-glucopyranosyl bromide (108)



108

The TMSET glycoside **106** (3.00 g, 3.91mmol) was dissolved in dichloromethane (25 mL), CF_3COOH (25 mL) was added at 0 °C, and the mixture was stirred for 30 min. Toluene (100 mL) was added and then removed under vacuum. A second portion of toluene (60 mL) was added and removed. The residue was then dissolved in pyridine (30 mL) and acetic anhydride (20 mL). After 3 h, the solution was poured into ice water and extracted with CH_2Cl_2 (300 mL) twice. The CH_2Cl_2 solution was washed with 1N HCl, H_2O, and saturated $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated, affording compound **107.** Crude **107** was then treated with 45 mL 33% HBr in acetic acid and stirred at 0 °C for 45 min and then poured into ice water and extracted with CH_2Cl_2 (2×200 mL), washed with H_2O, saturated $NaHCO_3$ solution, dried (Na_2SO_4), filtered and concentrated, affording bromo-sugar **108** (2.26 g, 79%). $[\alpha]_D$ + 83.6° (c 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 6.79 (d, 1H, J = 4.0 Hz, H-1), 6.18 (t, 1H, J = 10.0 Hz, H-3), 5.44 (t, 1H, J = 10.0 Hz, H-4), 5.25 (dd, 1H, J = 10.0 Hz, 4.0 Hz, H-2), 4.68 – 4.64 (m, 2H, H-5, H-6a), 4.67 (d, 1H, J = 12.0 Hz, $OCOOC\underline{H}_2Cl_3$), 4.60 (dd, 1H, J = 12.0 Hz, 5.0 Hz, H-6b), 4.53 (d, 1H, J = 12.0 Hz, $OCOOC\underline{H}_2Cl_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.88, 165.23, 165.13, 153.12, 133.84, 133.56, 133.40, 130.09, 129.87, 129.82, 129.77, 128.65, 128.54, 128.44, 128.23, 93.83, 86.35, 76.92, 76.85, 72.16, 72.01, 71.31, 70.33, 61.42, 55.64. HRMS (ESI): Calc. for Na [M+Na^+] $C_{30}H_{24}BrCl_3O_{10}Na$ 750.9511, observed 750.9499.

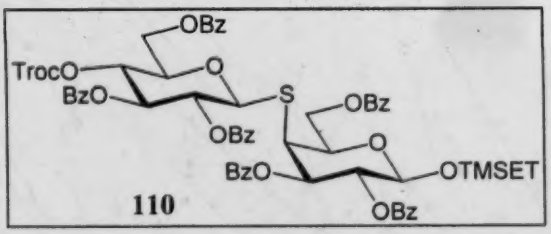
2,3,6-tri-O-benzoyl-4-O-trichloroethoxycarbonyl-1-S-acetyl-1-thio-β-D-glucopyranose (109)



109

Glucopyranosyl bromide **108** (2.00 g, 2.74 mmol) was dissolved in dry DMF under Ar.. Potassium thioacetate (936 mg, 8.22 mmol) was added and the mixture was stirred at rt for 4 h. The residue was diluted with CH_2Cl_2 (100 mL), washed with dilute HCl (1N, 50 mL), water (2 × 50 mL), dried (Na_2SO_4), concentrated and purified by column chromatography (hexane/ethyl acetate: 3:2) on silica gel, which gave title product **109** (1.69 g, 85%). $[\alpha]_D$ + 42.7° (c 1.0, $CHCl_3$); ^1H *NMR (600 MHz, $CDCl_3$)* δ 8.05 – 7.31 (m, 15H, Ar), 5.85 (t, 1H, J = 9.0 Hz, H-3), 5.25 (dd, 1H, J = 10.0, 8.5 Hz, H-2), 5.54 (d, 1H, J = 10.0 Hz, H-1), 5.36 (t, 1H, J = 10.0 Hz, H-4), 4.61 (d, 1H, J = 12.0 Hz, $OCOOC\underline{H}_2Cl_3$), 4.58 (dd, 1H, J = 12.5, 4.0 Hz, H-6a), 4.52 (dd, 1H, J = 12.5, 5.0 Hz, H-6b), 4.44 (d, 1H, J = 12.0 Hz, $OCOOC\underline{H}_2Cl_3$), 4.68-4.64 (m, 1H, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.95, 165.96, 165.23, 164.95, 153.10, 133.49-128.38, 93.86, 80.65, 76.12, 73.82, 69.69, 62.14, 30.80. HRMS (ESI): Calc. for Na $[M+Na^+]$ $C_{32}H_{27}Cl_3O_{11}SNa$ 747.0237, observed 747 .0240.

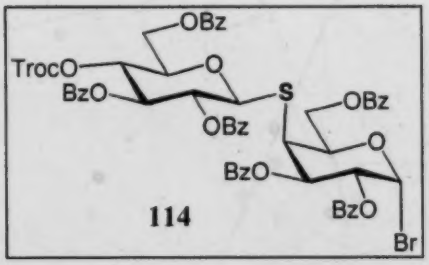
2-(Trimethylsilylethyl S-(2,3,6-tri-O-benzoyl-4-O-trichloroethoxycarbonyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-4-thio-β-D-galactopyranoside (110)



110

A mixture of thioacetate **109** (1.50 g, 2.07 mmol) and freshly prepared triflate **105** (1.65 g, 2.27 mmol) in dry DMF (50 mL) was stirred at –20 °C for 30 min. Diethylamine (5 mL) was then added under Ar atmosphere. The reaction mixture was stirred for a further 1 h below 0 °C then allowed warming to rt. Diethylamine was removed under vacuum. The residue was diluted with CH_2Cl_2, washed with dilute HCl (1N, 50 mL), water (3 × 300 mL), dried over Na_2SO_4, and concentrated. Column chromatography (hexane/ethyl acetate: 3:1) gave 1,4-*S*-linked disaccharide **110** (1.28 g, 49%). $[\alpha]_D$ + 42.3° (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 – 7.10 (m, 30H, Ar), 5.64 (dd, 1H, J = 10.0, 8.0 Hz, H-2), 5.52 (dd, 1H, J = 10.0, 2.3 Hz, H-3), 5.51 (dd, 1H, J = 9.5 Hz, H-3'), 5.44 (t, 1H, J = 10.0 Hz, H-2'), 5.28 (t, 1H, J = 10.0 Hz, H-4'), 5.04 (d, 1H, J = 10.0 Hz, H-1'), 4.67 (m, 3H, H-1, H-6a, H-6b), 4.59 (d, 1H, J = 12.0 Hz, OCOOC$\underline{H_2}Cl_3$), 4.53 (dd, 1H, J = 12.5, 2.6 Hz, H-6a'), 4.48 (dd, 1H, J = 12.5, 4.0 Hz, H-6b'), 4.43 (d, 1H, J = 12.0 Hz, OCOOC$\underline{H_2}Cl_3$), 4.25 (t, 1H, J = 6.0 Hz, H-5), 4.03 (dd, 1H, J = 4.5, 1.5 Hz, H-4), 3.93 (m, 1H, OC$\underline{H_2}CH_2SiMe_3$), 3.68 (dt, 1H, J = 10.0, 3.5 Hz, H-5'), 3.56 (m, 1H, OC$\underline{H_2}CH_2SiMe_3$), 0.08 (m, 2H, OCH$_2\underline{CH_2}$SiMe$_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.94, 165.83, 165.27, 165.23, 164.88, 152.95, 133.92-128.24, 101.20, 93.85, 82.82, 75.60, 74.07, 73.76,73.13, 72,58, 71.47,70.48, 67.36, 64.80, 61.93,17.85, -1.58. ESI-MS Calc. for $C_{62}H_{59}Cl_3O_{18}SSiNa$ [M+Na$^+$] 1279.2, observed. 1279.7, isotope intensities for Cl_3 correct.

S-(2,3,6-tri-O-benzoyl-4-O-trichloroethoxycarbonyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-4-thio--β-D-galactopyranosyl bromide ***(114)***

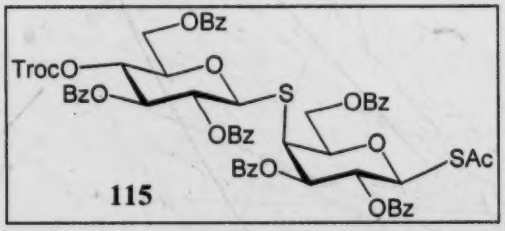


114

The TMSET glycoside **110** (1.20 g, 0.95 mmol) was dissolved in dichloromethane (50 mL), CF_3COOH (25 mL) was added at 0 °C, and the mixture was stirred for 30 min. Toluene (100 mL) was added and then removed under vacuum. A second portion of toluene (60 mL) was added and removed. The residue was then dissolved in pyridine (30 mL) and acetic anhydride (20 mL). After 3 h, the solution was poured into ice water and extracted with CH_2Cl_2 (300 mL) twice. The CH_2Cl_2 solution was washed with 1N HCl, H_2O, and saturated $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated, affording compound **113.** Crude **113** was then treated with 25 mL 33% HBr in acetic acid and stirred at 0 °C for 45 min and then poured into ice water and extracted with CH_2Cl_2 (2×200 mL), washed with H_2O, saturated $NaHCO_3$ solution, dried (Na_2SO_4), filtered and concentrated, affording bromosugar **114** (1.16 g, 78%). $[\alpha]_D$ + 85.1° (c 1.0, $CHCl_3$); ^1H *NMR (500 MHz, $CDCl_3$)* δ 8.10 – 7.20 (m, 30H, Ar), 6.78 (d, 1H, J = 4.0 Hz, H-1), 5.84 (dd, 1H, J = 10.0, 4.0 Hz, H-3), 5.51 (t, 1H, J = 9.5 Hz, H-3'), 5.43 (t, 1H, J = 10.0 Hz, H-2'), 5.41 (d, 1H, J = 10.0 Hz, H-2), 5.28 (dd, 1H, J = 10.0, 4.0 Hz, H-4'), 4.93 (d, 1H, J = 10.0 Hz, H-1'), 4.86 (dt, 1H, J = 5.0, 2.0 Hz, H-5), 4.64 (m, 2H, H-6a, H-6b), 4.61 (d, 1H, J = 12.0 Hz, OCOOC$\underline{H_2}Cl_3$), 4.48 (d, 2H, J = 4.0 Hz, H-6a', H-6b'), 4.45 (d, 1H, J = 12.0 Hz, OCOOC$\underline{H_2}Cl_3$), 4.12 (dd, 1H, J = 4.0, 2.0 Hz, H-4), 3.68 (dt, 1H, J = 10.0, 4.0 Hz, H-5'). ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.86, 165.78, 165.54, 165.24, 165.18, 165.16, 152.95,

134.18-128.39, 93.82, 88.60, 83.19, 75.75, 73.59, 73.11, 71.51, 70.78, 69.27, 64.77, 61.87, 46.18, 29.68. ESI-MS Calc. for $C_{57}H_{46}BrCl_3O_{17}SNa$ [M+Na^+] 1241.1, observed. 1241.5, isotope intensities for Cl_3 correct.

S-(2,3,6-tri-O-benzoyl-4-O-trichloroethoxycarbonyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-1-S-acetyl-1,4-dithio-β-D-galactopyranose ***(115)***

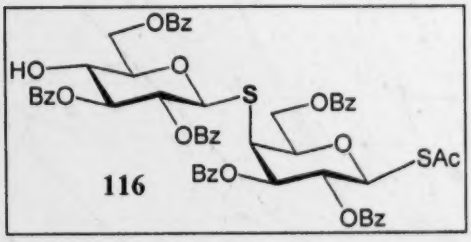


115

Compound **114** (1.10 g, 0.90 mmol) was dissolved in dry DMF under Ar. Potassium thioacetate (308 mg, 2.70 mmol) was added and the mixture was stirred at rt for 4 h. The residue was diluted with CH_2Cl_2 (100 mL), washed with dilute HCl (1N, 50 mL), water (2 × 50 mL), dried (Na_2SO_4), concentrated and purified by column chromatography (hexane/ethyl acetate: 3:2) on silica gel, which gave title product **115** (887 mg, 81%). $[\alpha]_D$ + 59.1° (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 – 7.20 (m, 30H, Ar), 5.73 (t, 1H, J = 10.0 Hz, H-2), 5.60 (dd, 1H, J = 10.0, 4.0 Hz, H-3), 5.48 (t, 1H, J = 10.0, 8.0Hz, H-3'), 5.46 (t, 1H, J = 10.0 Hz, H-1), 5.42 (d, 1H, J = 10.0 Hz, H-2'), 5.24 (t, 1H, J = 10.0 Hz, H-4'), 4.96 (d, 1H, J = 10.0 Hz, H-1'), 4.63 (dd, 1H, J = 12.0, 4.0 Hz, H-6a), 4.58 (d, 1H, J = 12.0 Hz, OCOOC$\underline{H}_2Cl_3$), 4.56 (d, 1H, J = 12.0, 6.0 Hz, H-6b) 4.46 (d, 2H, J = 3.4 Hz, H-6'), 4.42 (d, 1H, J = 12.0 Hz, OCOOC$\underline{H}_2Cl_3$), 4.37 (dt, 1H, J = 4.0, 2.0 Hz, H-5), 4.08 (dd, 1H, J = 4.0, 2.0 Hz, H-4), 3.67 (dt, 1H, J = 10.0, 4.0 Hz, H-5'). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.06, 165.96, 165.79, 165.59, 165.27, 165.25, 164.93, 152.93, 134.03-128.19, 125.27, 93.83, 82.95, 81.10, 77.78, 75.65, 74.92, 73.68, 73.90,

71.49, 68.11, 65.11, 61.91, 30.73. ESI-MS Calc. for $C_{59}H_{49}BrCl_3O_{18}S_2Na$ [M+Na$^+$] 1237.1, observed. 1237.5, isotope intensities for Cl_3 correct.

S-(2,3,6-tri-O-benzoyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-1-S-acetyl-1,4-dithio-β-D-galactopyranose ***(116)***

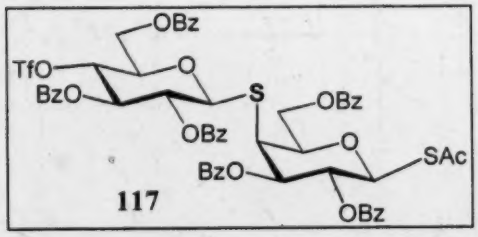


116

To a solution of **115** (800 mg, 0.66 mmol) in acetic acid (20 mL) was added Zn powder (3.70 g, 66 mmol), and the mixture was stirred at room temperature for 24 h and then filtered, concentrated. A solution of the residue in CH_2Cl_2 (100 mL) was washed with water, saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, concentrated and purified by silica gel chromatography (toluene/ethyl acetate: 10:1) to give **116** (577 mg, 84%) as a solid. $[\alpha]_D$ + 69.3° (c 1.0, $CHCl_3$); ^{1}H *NMR (500 MHz, $CDCl_3$)* δ 8.10 – 7.20 (m, 30H, Ar), 5.74 (t, 1H, J = 10.0 Hz, H-3), 5.61 (dd, 1H, J = 10.0, 4.0 Hz, H-2), 5.48 (d, 1H, J = 10.0 Hz, H-1), 5.37 (t, 1H, J = 10.0 Hz, H-2'), 5.18 (t, 1H, J = 10.0 Hz, H-3'), 4.89 (d, 1H, J = 10.0 Hz, H-1'), 4.68 (d, 1H, J = 12.0 Hz, H-6a'), 4.64 (dd, 1H, J = 12.0, 4.0 Hz, H-6a), 4.61 (dd, 1H, J = 12.0, 7.0 Hz, H-6b), 4.48 (d, 1H, J = 12.0 Hz, H-6b'), 4.37(m, 1H, H-5), 4.12 (d, 1H, J = 4.0 Hz, H-4), 3.78 (d, 1H, J = 9.5 Hz, H-4'), 3.42 (m, 1H, H-5'). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.16, 167.05, 166.87, 166.07, 165.62, 165.44, 164.97, 133.92, 133.41, 133.38, 133.26, 133.04, 129.98, 129.87, 129.85, 129.76, 129.73, 129.34, 129.07, 129.02, 128.97, 128.90, 128.78, 128.59, 128.48, 128.46, 128.39, 128.36, 128.30, 128.21, 82.71, 81.07, 78.63, 77.86, 74.98, 71.26, 69.00, 68.19, 65.38,

62.91, 46.42, 30.73. ESI-MS Calc. for $C_{56}H_{48}O_{16}S_2Na$ [M+Na$^+$] 1063.2, observed. 1063.2.

S-(2,3,6-tri-O-benzoyl-4- O-trifluoromethanesulfonyl-β-D- glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-1-S-acetyl-1,4-dithio-β-D-galactopyranose ***(117)***



117

To a mixture of **116** (200 mg, 0.19 mmol) and pyridine in CH_2Cl_2 (40 mL) was added Tf_2O (100 μL, 0.59mmol), and the mixture was stirred 0 °C for 3 h. The reaction solution was washed with water, saturated aqueous $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered, concentrated and gave **117** (223 mg, quant.) as a clear syrup. $[\alpha]_D$ + 58.6° (c 1.2, $CHCl_3$); ^{1}H *NMR* (500 MHz, $CDCl_3$) δ 8.10 – 7.30 (m, 30H, Ar), 5.72 (t, 1H, J = 10.0 Hz, H-2), 5.58 (t, 1H, J = 10.0 Hz, H-3'), 5.57 (dd, 1H, J = 10.0, 5.0 Hz, H-3), 5.44 (d, 1H, J = 10.0 Hz, H-1), 5.39 (t, 1H, J = 10.0 Hz, H-2'), 5.23 (t, 1H, J = 10.0 Hz, H-4'), 5.01 (d, 1H, J = 10.0 Hz, H-1'), 4.74 (dd, 1H, J = 12.0, 2.0 Hz, H-6a'), 4.57 (dd, 1H, J = 12.0, 4.0 Hz, H-6a), 4.52 (dd, 1H, J = 12.0, 6.5 Hz, H-6b), 4.37 (dd, 1H, J = 4.0, 2.0 Hz, H-5), 4.36 (dd, 1H, J = 12.0, 4.0 Hz, H-6b'), 4.04 (dd, 1H, J = 4.0, 2.0 Hz, H-4), 3.70 (dt, 1H, J = 10.0, 4.0 Hz, H-5'). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.99, 167.95, 165.57, 165.52, 165.16, 165.09, 164.96, 134.04-128.30, 82.98, 81.08, 78.52, 77.76, 75.49, 74.91, 72.65, 71.63, 67.99, 65.16, 61.37, 46.62, 30.73. ESI-MS Calc. for $C_{57}H_{47}F_3O_{18}S_3Na$ [M+Na$^+$] 1195.2, observed. 1195.1.

Chapter 6

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